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THE TIME OF ACTION OF GENES, AND ITS BEARING ON SOME EVOLUTIONARY PROBLEMS

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ANY classification of variations must at present be provisional, largely because of the extremely limited sources of information which we possess. To take two simple examples, the white flowers on dark-stemmed races of *Primula sinensis* contain flavone. This gives a bright or faint yellow color with ammonia, according as the plant does or does not carry a gene B which modifies anthocyanin color when anthocyanin is present. If our vision extended far enough into the ultra-violet to reach the flavone absorption bands, it would be possible to distinguish these whites without using ammonia. In the same plant a gene R, apparently by causing an acidity of the cell sap greater than that found in rr plants, converts blue flowers into red, the anthocyanin acting as an indicator, but produces no visible effect in the absence of anthocyanin. A genetically minded caterpillar, which regarded the taste of the petals as more interesting than their color, would (one may suppose) readily detect the gene R in white-petalled plants, just as we classify cherries into sweet and sour by their taste. It is highly probable that many morphological differences between varieties are caused by chemical differences appearing earlier in the life-cycle, which could be detected by suitable methods, so that the stage in the life-cycle at which a character appears is necessarily a function of our means of observation. Indeed with a

sufficiently fine technique it would be theoretically possible to observe the genes in the nucleus of any cell from any organ, except where some of the chromatin is eliminated in somatic cells. Nevertheless, we are justified in classifying genes by their effects observable with our present technique.

Any chemical or morphological classification would be very complex were it possible. But a classification in time simplifies matters, because variations can be arranged in a nearly (though not quite) linear order by the time of their appearance in the life-cycle, and in another order by the time of their disappearance. A given gene (and throughout this paper what is said of genes may be applied, *mutatis mutandis,* to other nuclear, and sometimes cytoplasmic, structures concerned in inheritance) may affect any six distinct organisms:

(1) The gamete carrying it.
(2) The zygote carrying it.
(3) The endosperm carrying it.
(4) The mother (not necessarily carrying it) of the zygote carrying it.
(5) A gamete (not necessarily carrying it) formed by a zygote carrying it.
(6) A zygote (not necessarily carrying it) produced by a mother carrying it.

This list is not of course exhaustive. Thus in metazoa with alternation of generations, such as the Anthozoa or Aphididae it may be expected that the action of certain genes will be confined to one of these generations. We could also distinguish genes according as they affect one or both sexes of zygote, or genders of gamete.

There are still further possibilities. A variation in the composition of milk might be detected by its effects on foster-children, a variation in the blood-corpuscles by the effect of injecting it into another individual, and so on. However, in principle it would seem that all such variations could be detected by other methods. But genes (or sets of genes) are known which can only be detected by their action on one or another of the six

organisms listed. It is further convenient to subdivide genes acting on the zygote by the time at which their effects are evident. We can then classify the genes of higher plants and animals according to Table I.

TABLE I

Designation	Action in plant	Action in animal
G	Gametophyte and gametic	Gametic
E	Endospermal	
MZ	Affecting maternal zygote	Affecting maternal zygote
Z1	Embryonic	Embryonic
Z2	Affecting seedling	Larval or late embryonic
Z3	Affecting immature plant, or non-productive organs of mature plant	Affecting immature animal, or characters of mature animal not connected with reproduction
Z4	Affecting reproductive organs (diploid tissue only) flowering time, etc.	Affecting reproductive organs or behavior, or secondary sexual characters
Z5	Affecting maternal seed and fruit structures	Affecting egg-shell, egg-white or other maternal egg or uterine structures
DG	Delayed gametophytic (affecting gametes, not necessarily carrying them, borne by zygotes carrying them)	Delayed gametic
DZ	Delayed zygotic (affecting zygotes, not necessarily carrying them, produced by mothers carrying them)	Delayed zygotic

The different stages in the zygote are of course arbitrary, and sharp distinctions can not be drawn. A different classification would be desirable in the Bryophyta, and perhaps even in the Pteridophyta. Thus Wettstein ('24) was able to observe the effects of one of four genes in *Funaria hygrometrica* in the protonema stage, of others not till later. So if we were dealing with Bryophyta we should want to break up group G into several subgroups. The same applies to the genes of the Protista and Thallophyta. Examples will now be given of the different types of gene.

G. Besides the above-mentioned genes in mosses, certain genes of the higher plants affect only gametes which carry them. Such are the genes which determine the type of polysaccharide in the endosperm of *Oryza* (Parnell, '21) and *Zea* (Brink and Abegg, '26). In each case the dominant form has ordinary starch, the recessive ("glutinous" and "waxy") contains a polysaccharide of different physical properties and giving a red color with iodine. The pollen grains contain the same polysaccharide as the endosperm in pure lines. But in heterozygous plants half the pollen grains contain each type of polysaccharide, and there can be little doubt that the type is determined by the gene carried by the pollen grain concerned. Parnell found that the immature pollen grains immediately after meiosis contain no starch. This develops gradually, under the influence of the particular gene carried by the grain. Similarly, in some but not all species, a pollen grain borne by a zygote with irregular meiosis attains to a size roughly proportional to the numbers of its chromosomes.

The genes determining membership of an intrasterile group in such plants as *Nicotiana* (East and Mangelsdorf, '26) fall into this class, and also into class Z4. Genes falling into class G only are indeed rather rare, but include the gene Ga for pollen tube growth-rate in *Zea Mays* (Mangelsdorf and Jones, '27). I do not know of any single gene in class G which affects megaspore characters. It is not yet clear whether the effects of Renner's "complexes" in *Oenothera,* which influence both microspores and megaspores, are due to single genes or gene groups. It is a noteworthy fact that no genes of this class are known in animals. Muller and Settles ('27) showed that the viability of the spermatozoa of *Drosophila* did not depend on genes either in the X-chromosome or in a section of the second chromosome which is essential for the life of the zygote. It is, moreover, known that apyrene spermatozoa may be fully motile, and it seems unlikely that the chromatin of a

spermatozoon has any relation other than a purely mechanical one to its other constituents. Doubtless this difference between higher animals and plants is due to the fact that in the latter the gamete represents a suppressed generation, which still preserves its own specific physiology. The difference is important from the evolutionary point of view because it implies that certation between gametes, which is an important type of natural selection in plants, is relatively unimportant in animals. It is also probably one reason why no animal is known whose genetics correspond to those of *Oenothera*.

E. Numerous genes are known affecting the endosperm in *Zea Mays*. Some, *e.g.*, "waxy," also fall into class G. Others, such as the gene for carotene (provitamin A) formation, are not known to act except in the endosperm. These genes have, of course, no analogies in animals, though possibly genes may be found controlling the structure and function of the tissue formed from the polar bodies in such forms as *Litomastix*.

MZ. This is the least studied group of genes with which we have to deal. Metaxenia, *i.e.*, difference between the influence exerted by different kinds of pollen on diploid maternal tissue, is quite a well-known phenomenon. Thus Crane and Lawrence ('29) showed that in *Prunus* and *Rubus* drupes or drupels generally require the formation of an embryo, and abort if it perishes early enough. But the differences in embryonic development thus influencing the maternal tissue were due, not to single genes, but to abnormalities involving whole chromosomes. Nixon ('28) and Swingle ('28) showed that in *Phoenix dactylifera* the pollen had a considerable influence on the fruit, fruit size and seed size being correlated. Here the effect is more probably to be ascribed to genes, though no analysis has been made. Metaxenia is of course only part of the action of genes of group MZ. If the mother is heterozygous for genes of this group, we shall find variation in fruit size due to this fact even if pollen of a homozygote is used. Tschermak ('31) gives a bibliography of MZ and Z1 action in plants.

There can be little doubt that (although no MZ genes are known in animals) genes of the MZ type have been of extreme importance in mammalian evolution. The foetus has a great influence on the mother, which must have been evolved by successive steps, and until such genes have been observed in mammals a large chapter in their evolution will remain obscure. Thus the placenta is known to contain hormones capable of acting on the mother, but nothing is known as to their variation. Nor do we know whether genetic factors in the foetus may not transform it into a chorion-epithelioma which kills the mother.

Z1. This group includes a number of well-known plant genes, such as Mendel's gene-pairs for round and wrinkled, yellow and green, cotyledons in *Pisum.* Some characters show at this stage, and are confined to it (*e.g.,* round and wrinkled). Others persist through life (*e.g.,* the sap color gene in *Matthiola,* Frost, '28). In animals the situation is not so good. Some lethal genes are known which act at this stage, but they may be regarded as acting throughout the rest of the life-cycle, as adults bearing them are conspicuous by their non-existence. An example of a gene acting at this stage is Toyama's ('13) recessive gene for crimson serosa color and reddish larval head color in *Bombyx mori.* The importance of purely embryonic variation in evolution is clear when we consider the extreme divergence of the early embryonic forms of species in which the adults are fairly similar, such as *Lepus* and *Cavia.* We do not know if this is due to Z1 or DZ genes, or possibly to extranuclear plasmons. The study of genes whose action is confined to the Z1 stage in higher animals is an important but difficult field for future research. A beginning has been made by Gregory and Castle ('31). They showed that in the eggs of a race of large rabbits, the number of blastomeres at 40 hours after mating was $9.94 \pm .24$, in a small race $8.29 \pm .19$. In hybrids derived from the eggs of a large female and spermatozoa of a small male

the number was $8.44 \pm .40$. In this case it is clear that Z1 genes carried by the spermatozoon had an important influence on cleavage rate, which was not mainly determined by DZ genes.

Z2. Apart from lethals, and genes for leaf shape and chlorophyll which show at this stage and persist through life, genes of this class are not very common in the higher plants, where seedlings are on the whole more uniform than adults. There is a little evidence for heritable variation in cotyledon shape not due to genes whose action is apparent in the adult, in *Primula sinensis*.

In animals such as holometabolous insects with a well-marked larval stage, differences confined to this stage are not uncommon, *e.g.*, several genes for larval color in *Lymantria monacha* (Goldschmidt, '27).

Z3. This class includes the majority of genes studied in animals, and all those in plants which affect the leaf and stem. These latter may or may not influence the flower.

Z4. This group includes the genes for flower character in plants; and a good many animal characters, *e.g.*, intersexuality, milk yield and egg yield fall into it. Perhaps the genes governing feather characters in birds which depend for their expression on the sex hormones (*e.g.*, henny feathers in poultry) should properly be included here.

Z5. This is perhaps merely a sub-group of Z4, but assumes a special importance in plant genetics, owing to the importance of fruits. Genes of class Z5 interact with E, Z1, MZ, and possibly DZ genes. Thus the shape of peas is conditioned by Z5 genes such as "indent," which acts on the maternal seed coat, and to a greater extent by Z1 genes, such as "wrinkled," which act on the cotyledon; and the pod character is mainly determined by Z5 genes. On the other hand, in the cherry and date Z1 genes are relatively unimportant except in so far as they also fall into group MZ.

In animals Z5 genes are not so important, but for the sake of symmetry such characters as those of the egg-

shell and albumen, which depend on secretions of the oviduct, may be included here. In the evolution of the mammal a critical part was doubtless played by Z5 genes which interact with MZ genes in the foetus to determine a suitable response of the uterus to its contents. We have not, of course, the faintest idea which group played the principal part in the evolutionary process.

DG. Delayed gametic characters are not uncommon in plants. Sometimes a gene is only known to act on gametes, *e.g.,* the gene for round as opposed to oblong pollen in *Lathyrus odoratus.* Sometimes it acts at other periods in the life-cycle. *E.g.,* the gene S in *Primula sinensis* produces a short style and long anthers, and also large pollen grains. Usually these genes seem to act wholly or mainly on the pollen, but the "polymitotic" gene in maize (Beadle, '30) causes extra nuclear divisions in gametes of both genders.

It must be emphasized that these genes act on all the gametes of zygotes carrying them, whether or not they are present in the gametes concerned. In some cases, *e.g.,* where size or shape of the grains is affected, their action does not continue in grains which do not contain them. But the normal allelomorph of the polymitotic gene suppresses supernumerary divisions of gametes borne by heterozygotic plants, even when those gametes carry the polymitotic gene. It clearly has a lasting influence on the surrounding cytoplasm.

Lesley and Frost ('27) describe a gene L in *Matthiola incana* which is on the borderline between Z4 and DG. LL and Ll plants have normal meiosis, with short chromosomes during meiosis. ll plants have long chromosomes during this period. Philp and Huskins ('31) regard the extra length as due to a delay in the onset of prophase contraction. This gene also has a DG effect, in that ll plants produce an unusually large proportion of gametes with chromosomal irregularities. Chromosomes may be lost or fragmented, or extra chromosomes may enter gametes, giving rise to trisomics.

If, therefore, the cytology of ll plants were not known, l might be regarded as a recessive DG gene for "bad pollen."

No cases are known with certainty of DG genes in animals, though it should be possible to detect the action of some DZ genes in the unfertilized egg. Thus it would almost certainly be possible to ascertain the color of silkworm egg-yolks in the ovary before fertilization. Since, as we saw above, it appears that genes do not function in spermatozoa, it is probable that the main differences between spermatozoa of different species are determined by genes of this class.

DZ. Genes of this group do not appear to be known with certainty in plants, though Miss Pellew informs me that the size of adult *Pisum* plants depends to a considerable extent on seed size determined by the mother and not the father. However, in this species inheritable variation in seed size occurs among peas in the same pod, *i.e.,* some of the genes determining size fall into group Z1. We have then not only to reckon with the fact that, besides environmental effects, genes of both these classes determine seed size, but with the possibility that the same gene may fall into both groups Z1 and DZ. If this is so the genetics of seed size will prove exceedingly complicated.

In animals DZ genes are well known. The classical case in *Bombyx mori* was described by Toyama ('13) as "maternal inheritance." This phrase might be taken to cover extra-nuclear inheritance, in which all inheritance takes place through females only. The tendency of modern genetics is to describe genetical facts in terms of gene action rather than heredity. So possibly "delayed gene action" is a more lucid phrase than "maternal inheritance." Some of Toyama's characters, such as spindle-shape and roughness of egg-shell, are Z5 characters due to a purely maternal structure. Others, such as yellowness of yolk, associated with yellow blood in the mother, are presumably DG. Others, however, such as

blueness, are due to pigment formed in the serosa, a zygotic structure, but do not seem to be at all influenced by the father. Along with these occur Z1 genes such as that for crimson eggs.

Since then Boycott, Diver, Garstang and Turner ('31), have shown that dextrality and sinistrality in *Limnaea peregra* are DZ characters, presumably depending on the structure of the unfertilized egg. Redfield ('26) discovered a lethal DZ gene in the second chromosome of *Drosophila melanogaster* affecting female embryos much more than males.

Experiments on interspecific hybridization in fish and echinoderms show that the rate and type of cleavage is mainly conditioned by the cytoplasm. If so it is controlled either by plasmons (characters inherited outside the nucleus) or by DZ genes. The evidence from species crosses yielding fertile hybrids shows that on the whole plasmons are far less important than genes in determining early development, not only in species where the cytoplasm of the egg is obviously differentiated, as in molluscs, but also where this is not the case, as in echinoderms. In mammals and other viviparous animals some maternal genes doubtless have a DZ action on the embryo through the uterus, and it would be hard to distinguish them from DZ genes of the normal type.

The range in time covered by a single gene is a matter of some interest, though data are scanty, because only very striking differences in the gamete and embryo are noticed at all. Thus C in *Pisum* is necessary for anthocyanin formation in the axils, color in the petals, the pods, and also in the seed-coat, *i.e.,* ranges from Z3 to Z5. But its detection depends on the presence of other genes. It does not for example affect the axil unless a special gene is present, or the seed-coat unless one of several genes is present. Perhaps a suitable chemical technique might detect its presence at earlier or later stages.

Frost ('28) gives an example of a gene acting over more than a life-cycle, so that a Z1 gene of one generation

interacts with a DZ gene of its predecessor. BB and Bb *Matthiola incana* (provided the color genes C and R and a plastid gene W are present) have purple flowers, bb being red. BB plants have black seeds, and bb brown. On the whole a Bb plant selfed gives 3 black (BB and Bb) to 1 brown (bb) seeded. But among the black seeds a few give red-flowered (bb) plants. Clearly the main action of B is to blacken embryos carrying it. But it may have a slighter effect in blackening bb embryos borne by zygotes carrying it. Fortunately, B can be scored unambiguously on flower color, but if it only affected seed color the genetic analysis would be very difficult. We must be prepared to find characters presenting this difficulty. Hogben ('31) thinks that in man blue sclerotics are due to a dominant gene of this type.

From the point of view of this paper certain evolutionary problems appear in a rather new light. As a general rule the embryos of related organisms are more alike than the adults. This merely means that the genes which determine interspecific differences mostly come into action rather late in the life cycle. The gill-clefts of embryo fish persist throughout life. Those of *Amniota* are transformed or disappear. The genes responsible for this difference must be present throughout the life-cycle, but only come into action during the stage Z2. The opposite is occasionally the case. The adult forms of many animals resemble one another more than the larval forms. Examples are the larva of *Unio* as compared with that of related salt-water mussels, the larvae of *Culex, Chironomus* and *Corethra,* and so on. These presumably differ by genes whose action is mainly confined to stage Z2.

There has been a common tendency in evolution for development to accelerate, *i.e.,* for certain characters to appear progressively earlier in the life cycle. Thus in the *Ammonoidea* complicated types of suture line first appear in the adult, and then progressively earlier in development. This presumably means that the time of

first action of certain genes has tended to be pushed back from stage Z4 or Z3 to Z2 and Z1. Although the genes may have been somewhat modified in the course of ages, this seems a simpler hypothesis than that quite new genes arose which produced an effect similar to that of the ancestral genes, but at an earlier stage.

Another common tendency has been a retardation of certain characters relative to the life-cycle, so that originally embryonic characters persist in the adult. This is known as neoteny or sometimes paedogenesis. In a neotenic animal such as man many adult ancestral characters, *e.g.*, the straightening out of the cranial flexure, do not occur. Presumably the genes causing cranial flexure, which in most mammals are confined in their action to stages Z1 and Z2, act for longer in man. Or alternatively various genes which act in stage Z3 of most mammals have either been lost or never come into action in man.

These are of course well-known examples. But so far the existence of G, DG and DZ genes has been forgotten in their discussion. In acceleration Z3 genes come to act in stage Z2, in neoteny the opposite process occurs. The result is then the sudden appearance in an adult form of new characters, which have evolved in ancestral embryos. De Beer ('30) to whose book I am indebted throughout this paper, describes this process as clandestine evolution. I think that the same processes may have an even wider scope. G genes may come to act in stage Z1 and conversely. Z3 and Z4 genes may come to act in stages DG and DZ and conversely.

It is possible that some of the more mysterious of evolutionary phenomena may be explained in this way. Very early *Ammonoidea* such as the Devonian *Bactrites* were straight, and the inner whorls of its contemporary *Mimoceras* did not meet. These primitive forms were replaced by organisms with normally coiled shells. But in later periods of degeneration the shells of a number of genera were coiled during the early part of the life

cycle, but later uncoiled. Such were the Triassic *Choristoceras* and *Rhabdoceras,* the Cretaceous *Lytoceras* and *Bactrites.* The general tendency in Ammonoid evolution had been to push back the action of genes from Z3 to Z2, and so on. I suggest that the gerontic straightness of degenerate forms was due to the pushing back of DZ genes governing embryonic development into the Z4 and Z3 stages. The adult *Bactrites* formed an uncoiled shell for the same reason that its ancestors formed a straight protoconch. The same argument could be applied to other cases where, after a long evolutionary history of acceleration, embryonic characters appear in the later stage of life-history.

The opposite process of caenogenesis may also perhaps occur in three ways, of which only the first has apparently been recognized. New Z1 or Z2 genes may appear. New DZ genes may appear. And finally Z3, Z4 or DG genes may come to act in stage DZ. We should expect the last to happen in connection with a general process of retardation. To take an example, Garstang ('28) has suggested that gastropod asymmetry began as a larval adaptation. We know that it is largely conditioned by DZ genes. These may have first appeared as such. But they may also have at first been Z3 or Z4 genes responsible for relatively trivial asymmetry in the adult or its cells, whose action was retarded in relation to the life-cycle, and thus came to produce larval asymmetry.

Whereas the change in time of action of a gene from Z2 to Z3, or conversely, presumably has effects which can at least be roughly predicted, this is not in general true for the changes from Z3 to DG or DZ, and conversely. Such changes are likely to lead to violent evolutionary novelties. A G or DG gene responsible for a lipase or protease in the spermatozoon might render uterine implantation possible for extending its action to cover stage Z1 or DZ. A DZ gene for rapid growth might cause malignancy if it came to act in stage Z4, and so on. The possibilities of clandestine evolution have probably been underestimated.

It may be objected that the above point of view is somewhat superficial. A DZ gene for shell straightness in an Ammonite, if its action were accelerated, would come into action of some kind, it might be urged, in the oocytes of the ovary, but not in the somatic cells responsible for shell form. I doubt if this objection can stand. Presumably during the life-cycle the protoplasm of the cells in the germ-track passes through a cycle of chemically definable stages, each being associated with a particular pattern of metabolism, and each being to a large extent the cause of the next. It is, however, possible, within certain limits, to alter the timing of one event relative to others, just as the time of ignition in an internal combustion engine may be altered relatively to the piston stroke. The cells lying off the germ-track seem to remain fixed for a long time in one stage of the cycle. If, however, other processes were accelerated in relation to meiosis, this might mean that not only oocytes, but somatic cells, passed into a chemical condition which had hitherto only been found in the embryo.

Acceleration or retardation can occur in two different ways. Thus in the case of acceleration the appearance or disappearance of one or many characters may occur at an earlier stage in the life-cycle in a descendant than in an ancestor. If only one or a few characters are concerned it is reasonable to suppose that the essential event was the acceleration of the action of the genes determining them (*e.g.*, the heart in birds or the horns in *Titanotheria*). But where the acceleration was general, as in certain lines of *Ammonoidea,* it is simpler to regard the process at work as a retardation of maturity in relation to other events. Such a retardation would of course allow DG and DZ genes to come into action before instead of after meiosis.

The reduction to vestiges of organs present in the adult ancestor and retained in the embryo (*e.g.*, notochord or pronephros) may often be due to acceleration. In some fish the notochord only ceases growing with the body as a whole. In other fish, and all *Amniota,* it ceases to grow

(apart from cases of chordoma) in an embryonic stage. This may be due to the presence of inhibitory genes, or merely to a pushing backwards of the time of activity of some gene or genes involved in notochord growth. In view of the large number of other vestigial organs which have shared the notochord's fate, the latter hypothesis is perhaps simpler.

So with the retardation of the appearance or disappearance of characters. This is most usually part of the general phenomenon of neoteny, which can be regarded as due to accelerated maturity in some cases (paedogenesis) and general retardation of structural development in relation to maturity in others (*e.g.*, man). It probably occurs also for individual characters in the absence of any general neoteny, but I do not know of any clear examples of this.

In the evolutionary speculations of the last century hypermorphosis played an important part. The descendant was supposed to go through all the stages of its ancestor, and a final stage in addition. As de Beer ('30) and others have pointed out, many of the alleged examples of this process can be equally well explained by deviation. From the genetic point of view such a process involves one of two events. Either a large number of new genes must come into being which act in stage Z3 or Z4, and simultaneously maturity must be delayed; or, by a general acceleration, DG and DZ genes must come to act in stages Z3 or Z4. The former process is a complex one needing many simultaneous adjustments. It is difficult to believe that it has occurred very often. The latter would be simpler, but might lead to racial "second childhood."

Underlying all the above speculations is the tacit assumption that the same process may accelerate or retard the appearance or disappearance of the activity of a large number of genes at once. I believe that this is justifiable. In many cases genes seem to act, not directly, but by producing enzymes. Whether these latter can be effective depends on circumstances. For example, catalase is es-

sential for the well-being of bacteria in presence of oxygen, but in its absence forms with and without catalase may do equally well. Koller ('30) found that rabbits with the gene E^p produced more (or a more resistant) certain lines of *Ammonoidea,* it is simpler to regard the dioxyphenylalanine oxidase than EE rabbits. But the question whether or not this gene will have any visible effect depends on the presence or otherwise of the inhibitor G, which produces little effect on rabbits with E^p. Now enzymes depend particularly on the pH and salt content of their medium. The latter at any rate often varies greatly during development. The growing tadpole absorbs proportionately much more water than salts from its environment. This process will affect a whole group of enzymes (*e.g.,* amylase, fumarase and catalase) and have very little effect on others. Thus a change in permeability due to a single gene would affect the time of action of many others. The same substance may be required for several quite separate processes, *e.g.,* glutathione for oxidation (Hopkins and Elliott, '31) proteolysis (Waldschmidt-Leitz, Purr, and Balls, '30), and starch hydrolysis (Pringsheim, Borchardt, and Hupfer, '31). So the biochemical effects of one gene may accelerate or retard the action of a whole group of others.

Equally important is the fact that at least in vertebrates the development of many characters is conditioned by hormones, *e.g.,* thyroxin and various gonadic secretions. So several processes exist by which the time of action of a number of genes could be accelerated or retarded simultaneously.

The gradual acceleration or retardation of a number of genes will lead to orthogenetic evolution. In many cases, as Goldschmidt ('27) has pointed out, the sooner a gene starts work, the more it can do. So that selection for a character will not merely cause the spread through a population of genes directly causing that character, but of genes accelerating their action. These latter will probably accelerate the action of other genes as well, leading to apparently useless evolution.

Two types of selection can be mentioned which probably cause general acceleration and retardation, respectively. When a number of embryos or larvae are competing in a limited area, *e.g.*, embryos in a mouse uterus, or seedlings from seeds dropped by the same tree, rapid growth will commonly be of great selective value, and the slower growing individuals will be weeded out. There will be a tendency to cut short the period of intense competition, and to push back the first appearance of characters as early as possible. So Z2 genes will begin to act in stage Z1, Z3 in Z2 and so on. This will react on the adult form both by giving certain genes longer to act, and possibly by accelerating the action of DG or DZ genes.

On the other hand, where a larva or embryo is well adapted to its surroundings, and can go on growing in relatively slight danger, there will be a tendency to prolong the embryonic phase. Examples may be found in the human embryo, which rarely suffers from twin competition, and in a savage country may well be safer than a new-born baby, or the aquatic larvae of many insects, which are well adapted to their surroundings, and do not generally compete directly with one another. In such cases the appearance of adult characters may be delayed. Z2 genes will not begin to act till stage Z3, and so on. There will be a tendency to neoteny, and possibly a retardation of Z3 and Z4 genes to act in stages DG or DZ. This can of course be counterbalanced by intercalating, as in the holometabolous insect, a period of catastrophic metamorphosis, during which genes act with very great speed. This again may lead to unpredictable results. Genes particularly adapted to the chemical environment of the chrysalis will assume particular importance. Others will cease to have selective value. As the metamorphosis becomes more and more pronounced there will probably be a tendency to select, on the one hand, genes acting only at this time, on the other, genes whose action abruptly ceases then. The more the time of action of genes can be limited, the more the possibility of combining adaptation in the larva and adult.

In general the more limited is the period of action of a gene, the more unalloyed will be its benefits if it is useful at a certain period. There is no reason to suppose that the same type of polysaccharide is desirable in pollen and endosperm in maize, but so long as one gene controls both, the adaptation of the endosperm will be subordinated to that of the pollen grain, or conversely. The greater the difference in cell chemistry at different stages of the life-cycle the greater should be the possibility of limiting gene action. It would therefore seem that within limits natural selection will tend to make life-cycles more and more variegated, as every increase in complexity will increase the possibility of fixing the time of action of genes. Clearly there are limits to the process, but it may be a partial explanation of some of the apparently useless complexities of biology, such as the tendency of parasites to live in widely different hosts. We must consider the possibility that a liver-fluke is well adapted to its very different environments just because on changing from a diet of *Limnaea* to a diet of *Ovis* at a higher temperature, the chemical conditions in it are so changed as to allow a new set of genes to come into action, and it can thus possess two sets of genes, one acting in each host, and each capable of evolving in almost complete independence of the other.

Another way of fixing the time of action of genes is by elimination of part of the chromatin in somatic cells, as in *Ascaris* and *Miastor*. This method has, however, rarely been adopted. It must involve the localization together of all genes whose action is limited to an early stage of development, and are not essential later on. In view of the lack of correlation between the location of genes and their function, it is surprising that such elimination ever occurs, and intelligible that it is rare, although it is the mechanism which one would obviously adopt in designing a "synthetic animal."

We see, then, that the time of action of genes not only merits further study by the geneticist, but is essential for a detailed discussion of evolution.

SUMMARY

(1) Genes can be classified according to their time of action. Not only can they act in diploid zygotes, endosperms or gametes carrying them, but their action can be manifested in the following organisms which do not carry them: mothers of zygotes carrying them, and gametes or embryos borne by zygotes carrying them. Their time of action is therefore distributed over more than one life-cycle.

(2) It is contended that change in the time of action of genes has been an important factor in evolution, and that some cases of orthogenesis, including degeneration, can be explained by it.

(3) In an organism undergoing metamorphosis the adaptive efficiency of a gene depends on the limitation in time of its action.

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THE CONTROL OF THE CHROMOSOMES BY THE GENOTYPE AND ITS BEARING ON SOME EVOLUTIONARY PROBLEMS

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I. INDUCTIVE

THE primary postulate of genetics is that the properties of living organisms are the resultant product of the interaction of "genotype" and "environment," and on this assumption the study of their inheritance, variation and adaptation can be consistently developed. But it is not true of the properties of their chromosomes. The chromosomes have, it is believed, an individuality, a permanent structure, which can not be subject to the same control as that to which other organs are subject. This conclusion is forced on us from four directions. First, the original observations of Boveri on the continuity in the existence of chromosomes are supported by the great bulk of systematic observations: their form and number in each cell-generation are constant and their behavior is consequently predictable. Secondly, where changes are found to occur under experimental conditions they can nearly always be shown to arise from changes in the potentially permanent structure of the chromosome. These do not result from changes in genetic conditions but rather as accidents and actually themselves determine genetic changes (for predictions based on this assumption are regularly verified). Thirdly, the continual strengthening of the chromosome theory of heredity necessarily strengthens the belief in the permanence of the chromosomes, since heredity is merely a manifestation of permanence. Finally, the notion that there is something absolute about the properties of the chromosomes and that the relation between these properties and the genetic properties of the organism is therefore a

simple and direct one has been widely used as a basis of deduction and has therefore acquired the prestige of dogma.

But the permanent structure is internal to the chromosomes. And the question arises: is not a considerable variation in external form and behavior, such as would be genetically determined, compatible with strict permanence of internal structure? Evidence is beginning to show that such variation occurs. But the strength of the prevailing doctrine has often prevented the proper inference being drawn, and the isolated character of the observations has prevented their wider significance being seen. It will therefore be worth while to recall these observations and consider their meaning.

Our problem is to find out (i) to what extent changes in the properties of a chromosome are due to independent changes in the affected particles (*i.e.*, in permanent structure), (ii) to what extent they are due to the action of the chromosomes as a whole, the genotype, on their constituent parts and (iii) whether any third factor must be assumed. It must be borne in mind that, although the action of the genotype is customarily determined by observing the differences between two genotypes which are due to changes in certain of their constituent parts, a genotypic change will probably not be specific to the part affected. A structural change on the other hand will always be specific. Moreover, it will occur under two kinds of conditions, which can already be defined with moderate precision, *viz.:* (i) at random with regard to the position and type of change, whether fragmentation, translocation or other kind, and usually at any part of the life cycle but perhaps chiefly at meiosis. (ii) At the prophase of meiosis as a result of crossing-over between homologous parts of chromosomes which are associated with dissimilar parts. The first may be described as *primary,* the second as *secondary* structural changes since they can only arise from the occurrence of the first.

It follows therefore that the essential distinction between the results of a change in structure and in genotype is that the one is confined to the points at which it occurs, while the other is not so confined and is likely to be general in effect. We may then expect a difficulty to arise in applying this distinction when the organisms whose chromosome form and behavior are being compared are so remotely related that numerous structural differences may be supposed to have arisen between them. But when they are closely related, even though conditions of inheritance are not understood, we may hope to draw an unequivocal conclusion.

The evidence may therefore be classified, according to the stringency of the inference to be based on it, in five different categories, as follows:

(a) The difference is characteristic of races and is inherited as a Mendelian character (*e.g.,* chromosome contraction at meiosis in *Matthiola* or failure of meiosis in *Zea*).

(b) The difference arises through mutation in a known individual (*e.g.,* chromosome size in *Tradescantia* or parthenogenesis in *Rhabditis*).

(c) The difference is seen between the chromosomes of a hybrid and the same chromosomes in its parents (*e.g.,* chromosome size, length and constriction in *Crepis* and *Vicia*).

(d) The difference occurs between different stages in the development or parts of the body of an individual (*e.g.,* facultative parthenogenesis in *Allium odorum,* chromosome diminution in *Ascaris*) or in the life cycle of a species (*e.g.,* cyclic parthenogenesis in Aphides) while related species are constant in one form or the other.

(e) The difference occurs between related races, species or larger groups (*e.g.,* chromosome contraction in *Phragmatobia* and *Crepis,* compound chromosome formation and diminution in *Ascaris*).

While there can be little doubt of the cogency of the first three types of evidence, the last two may be thought insufficient. The type of inference is a rather new one although it has been used by Haldane ('31a) in analyzing variation in other organs from a similar point of view. The question, however, is simply this: can the differences involved be explained on an analogy by the recognized alternative of structural change? In my opinion, in the cases I am going to consider, they can not.

The following are examples of differences attributable to the subordination of the chromosomes to the genotype. The first four and the last are concerned equally with mitosis and meiosis, the rest are peculiar to meiosis.

(i) *Chromosome size.* (a) A triploid plant, a presumed hybrid between tetraploid *Tradescantia virginiana* with large chromosomes and a diploid species with chromosomes about one fifth the bulk (but corresponding in form) had chromosomes corresponding in bulk to the larger-chromosome parent. One bud on this plant had chromosomes one fifth this size at the pollen grain mitosis. Its resting nuclei were approximately one third the size. It was therefore concluded that the change was due to a genetic mutation determining the bulk of chromosomes and nucleus and that such a mutation distinguished the parent species (Darlington, '29c and unpublished). (b) A difference in the size of the autosomes in male and female *Sphaerocarpus* is perhaps due to a less pronounced change of the same kind (Lorbeer, '30). (c) In the hybrid *Vicia sativa* × *Vicia angustifolia* (Schweschnikowa, '29a) the chromosomes are smaller at mitosis and meiosis than in the parents. This difference must also be regarded as a genetic property. It is important to notice that this variation is not attended with any significant change in cell size.

Whether the changes of size genotypically determined are due to a multiplication of the specific hereditary molecules or to a greater dispersion of them is a question that need not be considered here.

(ii) *Chromosome Contraction.* (a) In *Phragmatobia fuliginosa* (Seiler, '25) two races occur with different degrees of linear contraction (lateral expansion) at metaphase of mitosis (maximum contraction occurs in both forms at meiosis). The degree of contraction is constant throughout the complement and in different nuclei in the higher organisms generally. The diameter of the chromatids at metaphase is a specific character. When, therefore, it is found as a varietal character and no difference can be found in any other respect between the varieties it can be regarded as genetically determined.

(b) Similarly, in *Melandrium album,* Breslawetz ('29) found two individuals with chromosomes contracted to one third their normal length, as though at meiosis. This property was constant and therefore genotypically determined.

(c) It may be concluded that a difference of this kind distinguishes *Crepis capillaris* and *C. neglecta,* for the chromosomes of the first are longer and of the second shorter in the hybrid between them than in the parental species (Navashin, '31b).

(d) Clausen ('31c) finds a reduced longitudinal contraction of the chromosomes at meiosis in an F_3 segregate from the cross *Viola tricolor* $\times$ *V. Orphanidis.* This must also be regarded as a genetically determined reduction of contraction, probably characteristic of mitosis, since it is seen at the second division (*cf. Matthiola*).

(iii) *Chromosome Constriction.* In various first generation *Crepis* hybrids a trabant, *i.e.,* a small distal part of a chromosome separated by a long constriction from the main body, characteristic of a parental chromosome was found to be fused with the main body of the chromosome. Here, although the difference is single, as though produced by structural change, the structure of chromosomes must be the same in a hybrid and in the parent and the change must therefore be genotypic. This means therefore that the genotypes of parent and hybrid (as in the *Vicia* case above) differed in their reaction to the con-

strictions of chromosomes (Navashin, '26, Hollingshead, '30b). The disappearance of the constriction is probably a symptom of increased longitudinal contraction of the chromosome.

(iv) *Chromosome Aggregation and Diminution.* The chromosomes in somatic cells of *Ascaris megalocephala* undergo the well-known changes of fragmentation and diminution. Comparison with related species shows that the fragmentation is probably a reversal to an ancestral condition. The properties by which the separate elements are held together permanently in the germ line and by which parts of them are lost in the body cells, must therefore be genetic. This view is strengthened by the observation by Seiler and Haniel ('21) of reversible fusion at a particular stage of the life cycle in the male of *Lymantria monacha.* The temporary fusion observed in this species has apparently become permanent in the related species *Phragmatobia fuliginosa.* The property of diminution in *Ascaris* and *Miastor* is similarly a genetic-developmental reaction.

(v) *Precocity of Meiosis.* In *Matthiola incana* a race occurs with chromosomes less contracted at meiosis than in the normal type. They look more like ordinary mitotic chromosomes, and they are liable to be irregular in pairing (Lesley and Frost, '27, Philp and Huskins, '31). This property has been attributed to a partial failure of precocity in the prophase which is supposed to determine the pairing of chromosomes at pachytene and the characteristic exaggerated contraction at the metaphase of meiosis (Darlington, '31b). It is characteristic of the metaphase of the first division; it is scarcely appreciable at later stages and absent in ordinary mitosis. This distinguishes it from the abnormality in *Viola.* Since the property behaves as a Mendelian recessive, there can be no doubt of its genetic basis.

The local suppression of meiosis in *Allium odorum* (Modilewski, '30), its cyclic suppression in *Phylloxera* (Morgan, '15) and other parthenogenetic organisms, and exceptional suppression in individuals of some species

(*e.g., Ascaris megalocephala bivalens,* Geinitz, '15) are probably due to the complete failure of pachytene pairing and the replacement of meiosis by mitosis. They may be taken to represent an exaggeration of the effect observed in *Matthiola.* They are certainly determined genotypically and not structurally, *i.e.,* by hybridity. It may be supposed that these genotypic effects approximately reverse the process by which meiosis originally arose from mitosis by a too-early beginning of the prophase (*v. infra*).

(vi) *Chiasma Formation.* Clones of *Fritillaria imperialis* occur with differences in the frequency with which chiasmata are formed in the paired chromosomes at the diplotene stage of meiosis (Darlington, '30c). The difference is characteristic of all the chromosomes and of all the bulbs of the clone; there is therefore no reason for assuming hybridity such as would reduce the possibility of forming chiasmata in one clone, or for assuming environmental differences such as might modify the frequency, and the difference can be attributed only to genetic factors.

The suppression of the pairing of chromosomes at meiosis in *Zea Mays* (Beadle, '30) since it does not affect the linear contraction of the chromosome, is perhaps due simply to a complete suppression of chiasma formation and not to suppression of pachytene pairing. The same is true of the failure of pairing in the male-sterile *Viola Orphanidis* (Clausen, '30) and in the hybrid *V. nana* $\times$ *V. arvensis* (Clausen, '31). The maize abnormality is determined, like the reduction and suppression of crossing-over in *Drosophila* (Gowen, '28; Bridges, '29) by a single recessive factor. On the chiasmatype hypothesis, differences in crossing-over frequency and distribution are analogous to similar variations in crossing-over. The suppression of chiasma-formation is probably an alternative means to failures of pachytene pairing of securing failure of reduction in parthenogenesis (*cf.* Beadle, '30).

(vii) *Distribution of Chiasmata.* In *Mecostethus gracilis* and *Fritillaria Meleagris* the chiasmata are localized in the neighborhood of the spindle attachment (McClung, '14, '28; Janssens '24; Newton and Darlington, '30). This is probably to be attributed to failure of pairing, partial or complete, in the distal segments of the chromosomes and this failure again to differential precocity (see below) preventing the pairing of parts of chromosomes which have divided before the rest (Darlington, '31b). Whatever its mechanism, localization, in regard to which species of *Fritillaria* differ, is a genetic property. Such a property has been found to control the distribution of crossing-over in *Drosophila* (Bridges, '29; *cf.* Darlington, '31a). It may be noted that the differences in distribution between the different chromosomes in *Drosophila* must clearly be structurally determined. They are perhaps related differences in the spacing of the hereditary material in its supporting framework—differences of which the visible signs are the constrictions.

Another property affecting chiasma distribution is interference (Haldane, '31b). It is probable that races differ genetically in this respect (Darlington and Janaki-Ammal, '32).

(viii) *Terminalization of Chiasmata.* Related species differ in the degree of terminalization of chiasmata (*e.g.*, in *Tradescantia,* Darlington, '29b), although as a rule large groups are fairly constant in this property: the Hemiptera, Lepidoptera, Tettigidae and the Onagraceae and Solanaceae, generally have a high degree of terminalization; the Acrididae and the Liliaceae, the Gymnosperms and many Leguminosae and Rosaceae have little terminalization. There is no evidence as yet of great differences within the species (but *cf. Fritillaria,* Darlington, '30). Nevertheless, amongst many conditions of terminalization that are now coming to light none have been found to account for the radical differences found between different pure species on any other than

a genetic basis. It must therefore be supposed that species differ in genetic conditions determining the degree of terminalization.

(ix) *Precocious Condensation of Chromosomes.* Although the property of differential condensation is almost entirely confined to the stage of meiosis and to chromosomes (actually sex chromosomes) which have the exceptional property of not pairing at this stage, there is evidence to show that the lack of pairing does not usually determine this behavior, but that a genetic-environmental reaction may do so.

Thus chromosomes may show differential condensation at pre-meiotic divisions (Brunelli, '10, '11; Boveri, '11 *et al.*). An unpaired X-chromosome which shows condensation in the testis does not show it in exceptional ovarian tissue in the male of *Perla marginata* (Junker, '23). Such differential behavior probably determines failure of pairing in the spermatogonia of *Rhabditis* (Boveri, *l.c.*). In these cases an environmental (developmental) condition determines the difference in behavior of particular chromosomes; thus the possibility of its being due to a structural difference or a lack of partner is excluded. If this may therefore be a genetic property we have an alternative explanation for its general correlation with failure of pairing. The condensation may be the cause, not the effect; for failure of pairing between chromosomes carrying the sex-factors, if determined in this way, would separate the corresponding chromosomes phylogenetically and thus permit of the origin of differences which would later in themselves prevent pairing (*v. infra*).

This differential property of certain chromosomes like that referred to above, of parts of chromosomes, is significant from another point of view. It is difficult to suppose that such differential behavior is a reaction of the specific hereditary material which is specialized for an entirely different function. The reaction therefore points to the existence of associated materials of non-

genetic function (such as are also responsible for the constrictions and other discontinuities referred to above). It therefore provides evidence for a third factor in chromosome behavior, *viz.*, properties of *accessory* materials, other than the essential hereditary materials, or unessential conditions of these materials in the chromosome.

(x) *Polarization of the Nucleus at Zygotene.* Little is known of the distribution of this property, owing to difficulties of observation at the early prophase of meiosis. Consequently no differences in regard to it are recognized between closely related forms. Of the two best known groups one, the Orthoptera, has regular polarization, the other, the Liliaceae, has none.

(xi) *Occurrence of a Diffuse Stage in the Prophase of Meiosis.* This property is widely distributed in animals but is never found in plants. It does not seem to affect the eventual behavior of the paired chromosomes and is not therefore of interest to us at present.

(xii) *Suppression of Mitosis, Nuclear Separation, Division of Chromosomes, etc.* Factors affecting the mitotic mechanism are well recognized and do not affect the present problem closely. Of genetic significance are those which must be supposed to determine:

(a) Syndiploidy in lizards and grasshoppers (Painter, '21; Eisentraut, '26) in *Brassica japonica* (Fukushima, '31) and in *Zea Mays* (Beadle, '30) where it is associated with genetic failure of meiosis.

(b) Fusion of nuclei after the second division or in an early segmentation division in many cases of diploid parthenogenesis with normal meiosis (*e.g., Rhabditis,* Bělař, '23). This property can arise as a mutation (P. Hertwig, '20).

(c) Supernumerary mitoses in the pollen grain of *Zea Mays* (Beadle, '31). This is proved to be a recessive character.

(d) Failure to form daughter nuclei regularly after meiosis in strains of *Triticum vulgare* (L. A. Sapehin,

'31) and in species of *Kniphofia* (Moffett, unpublished), a condition probably related to male sterility, which is determined by a mendelian factor in many species.

(e) Polarization of the embryo-sac in the *Caninae* roses, by virtue of which all unpaired chromosomes usually pass to one daughter nucleus (Täckholm, '22).

(f) The reduction of the X-chromosomes at the abortive meiosis in male-producing parthenogenetic aphides (Morgan, '15).

The supernumerary mitoses in *Zea Mays* seem to be of theoretical importance. The prophase of the first of these divisions begins before the chromosomes have divided, and at metaphase the chromosomes show an excessive linear contraction. In both these respects the division resembles meiosis. It can only resemble the meiosis of a haploid, and the occasional pairing at metaphase, by interstitial chiasmata, is therefore particularly significant. This is probably analogous to the pairing observed in haploid *Oenothera* by Emerson ('29). It may be supposed to result in both cases from the pairing of reduplicated segments in the hybrid set, which do not normally get a chance to pair on account of not being in the same linear sequence. The structure of the interstitial chiasmata suggests that the chromosomes have divided during the prophase, as at meiosis, and in the four or five divisions which rapidly succeed one another their halves must be supposed to separate in order to give as many as 32 cells. The genetic condition therefore seems to resemble that determining meiosis, with two differences: (1) It acts on haploid nuclei and therefore can not give regular reduction, (ii) it stimulates not two but a whole series of divisions.

The effects of environmental (and developmental) differences have already been considered in so far as they merely condition the expression of genetic differences. Other environmental differences have effects on the chromosomes as on other organs parallel to those of genetic differences—presumably because they are physio-

logically analogous. This parallelism is important because where it occurs it excludes the possibility of a structural basis for the variations concerned. Thus changes in temperature and application of various reagents will (i) increase the degree of prophase contraction of chromosomes (Delaunay, '30), (ii) suppress or modify the anaphase separation of chromosomes at mitosis and meiosis (Sakamura and Stow, '26; Nemec, '29, *et al.*). Other changes in external or developmental conditions will (i) change the amount of crossing-over (Plough, '17; Bridges, '29) (ii) change the frequency of chiasmata in a chromosome pair (*cf.* Darlington, '31b, Fig. 6) (iii) change the frequency with which chromosomes pair in a hybrid (Kihara, '29) and thus sometimes abolish pairing altogether locally (Meurman, '29, on *Ribes*).

Environmental changes have not yet been shown to influence (i) the bulk of chromosomes individually or as a complement at metaphase, (ii) the position of the attachment constriction, (iii) the formation of multiple chromosomes or their disintegration, (iv) the localization and terminalization of chiasmata. Their effects cover an especially narrow range as compared with those of genetic differences, presumably because they can operate less directly and less specifically on the chromosomes than on the gross structure of the organism. They merely provide one measure of the degree of elasticity of the mechanism. Environmental effects are significant in relation to evolutionary theory in one respect: unlike effects on the gross structure they are never of an adaptive character. The adaptations in chromosome behavior to be considered later are therefore especially cogent evidence against the Lamarckian hypothesis.

Note. The failure of the division of chromosomes, leading to their elimination and the formation of mosaics in *Drosophila* and Maize (Bridges, Stern, Stadler, *et al.*), are more probably due to structural peculiarities of the chromosomes concerned. Since the supernumerary chro-

mosomes of *Camnula* seem to show a special propensity for irregular distribution (Carroll, '20) their origin is similarly to be attributed to irregular division of one of the normal chromosome complement associated with a structural peculiarity.

These observations enable us to classify the sources of variation in chromosome behavior under four heads: (i) Changes in the permanent (hereditary) structure, (ii) changes in the genotype, (iii) changes in the "accessory materials" in the chromosomes, (iv) changes in the environment. The first and fourth controlling factors have long been recognized; the third can not yet be exactly estimated or even defined; but the second is a factor whose importance can now be considered.

II. Deductive

The importance of the inference of genetic control is twofold.

(i) *A distinction between genetic and structural causes of differences in chromosome form and behavior is necessary for deciding their evolutionary significance.*

The observations in question are of two kinds:

(a) Comparative observations of chromosomes at *mitosis* in related species and varieties: from these the relationship of the species is deduced. Clearly it is important to know whether a difference in the size of chromosomes is the result of the action of a single genetic factor or of the accumulation of structural changes each of which would have an important genetic effect. In the present state of our knowledge conclusions must be tentative, but it is easiest to suppose that the differences in chromosome size between *Nicotiana* and *Solanum,* between *Tradescantia virginiana, T. crassifolia* and *Cyanotis,* and between different sections of *Lactuca, Eschscholtzia* and *Campanula* are determined largely by genetic factors, while the difference between *Nicotiana alata* and *N. sylvestris,* between *Tradescantia virginiana* and *T. navicularis* and between different species of *Gryllus* (Ohmachi, '29) are determined by structural

changes—fragmentation or fusion (refer to Tischler, '31).

It is of course difficult to estimate the relative importance of structural and genotypic change in determining variation in the bulk of chromosomes in living organisms as a whole; but their relative range of effect may be tentatively estimated. Differences within a complement are wholly due to structural differentiation, since the same genotype controls the whole complement. The highest range found in a complement is in the relation of about 1:50 (*Muscari latifolium,* Delaunay, '29). The highest range between the *largest* chromosomes of two complements is found within a single order of Dicotyledons, the Droseraceae, and is between volumes in the proportion of 1:1000. This difference must be almost wholly genotypically controlled.

The two ranges are both effective ranges, and neither of them represents the theoretical maximum, which is necessarily much greater for structural change. But the comparison shows that in effective range genotypic control is probably the more important source of variation.

Note. The chromosomes of *Aesculus Hippocastanum* are one eighth the size of those of *Ae. pavia* and its section of the genus. In the presumed hybrid *Ae. carnea* the distinction is retained (Skovsted, '29). It can not therefore be determined by the genotype. Yet it seems impossible to suppose that so uniform a difference has arisen through structural change. Here then seems to be a second kind of evidence for accessory substances in the chromosomes as a third factor in their behavior.

(b) Observation of *meiosis* in hybrids: from these, on the assumption that the frequency of pairing is a measure of affinity, extensive studies of species-relationships have been carried out in *Triticum, Nicotiana* and other genera. But the pairing is only an indirect measure of the frequency of chiasma formation (Darlington, '31b), and this in turn is an unknown measure of the length of chromosome paired at pachytene and of the effect of genetic

factors. This length is conditioned by identity of the materials making up the chromosome and the identity of their arrangement—as shown by Dobzhansky's observations ('31) on the reduction of crossing-over in translocation heterozygotes (*cf.* Darlington, '31c). A frequent lack of relationship between the "affinity" of species and of their chromosomes is therefore to be expected, and is very often found (*cf.* Federley, '14).

It will be seen therefore that these observations of affinity, while of great theoretical interest, can not be applied to the special problem of phylogeny without a consideration at least of conditions of chiasma formation in the parents as well as in their hybrids. While the forms of bivalents (indicating the frequencies and distribution of their chiasmata) have been illustrated in *Triticum* and *Aegilops* hybrids with remarkable accuracy (Kihara, '29, *et al.*) there is no illustration whatever to show comparable conditions in their parents. A method of approach to this problem has been described elsewhere (Darlington, '31d).

(ii) *The assumption that chromosome form and behavior are subordinate to the genotype is the remaining postulate necessary for the establishment of a single inductive-deductive system in which all observations of chromosome form and behavior can be consistently arranged.*

With the assumption that chromosome form and behavior is subject to genotypic control its variation amongst species can be considered as adaptive. Adaptation must be assumed to be brought about by natural selection of variations on the Darwinian principle. (The Lamarckian hypothesis is excluded by the considerations given above, as well as on general grounds). The results of study from this point of view are indispensable to evolutionary theory, since the variations observed in some measure control such properties as sexual reproduction, sexual differentiation, parthenogenesis and polyploidy. The following may serve as examples.

(i) Small chromosome size is an adaptation to polyploidy, which has only reached a high development in the dicotyledons with small chromosomes. It is also necessarily a concomitant of small cell-size. The hereditary material can not be supposed to be essentially different in organization in different groups of insects and flowering plants, in accordance with the great size differences observed. The chromosomes of the Liliaceae and Orthoptera would be mechanically incompatible with the cells of the Rosaceae and Lepidoptera. Genetic variation may therefore be supposed to have led to adaptation of chromosome size to cell size.

Since cell size and chromosome size are independent in the sources of their variation, but associated in its expression, it is not possible to determine at once which is the limiting factor. But variation affecting cell-size is of universal occurrence. It has undoubtedly played a part in the origin of most polyploid species, since these have not the cell-size proportions that are found in new polyploid forms. Genotypic variation in chromosome-size, on the other hand, seems to be absent in many large groups (such as the Rosaceae and the Lepidoptera). Where it occurs it is usually found to lead to a wide range of sizes such as the three distinguishable in the Droseraceae and the Tradescantiae. Probably therefore we may look to genotypic variation in chromosome size as a controlling factor in cell-size variation.

(ii) Aggregation of small chromosomes is an adaptation which secures regular pairing without high chiasma frequency. High chiasma frequency may have two disadvantages, (a) on the chiasmatype theory it means high crossing-over (which will tend to reduce heterozygosis in a population when lethal or depressive mutations occur), (b) in the absence of terminalization (before metaphase) it leads to lagging of the paired chromosomes at anaphase with possible irregular disjunction where the bivalents differ. It is therefore interesting to notice that

this aggregation is only retained in *Ascaris* in those chromosomes which are to undergo meiosis. This point of view leads us to consider that fragmentation and diminution do not themselves determine the germ track but are parallel genetic-developmental effects to that determination.

(iii) High chiasma frequency (with high crossing-over on the chiasmatype hypothesis) secures the most rapid distribution and recombination of variations in a species. But unless combined with some degree of terminalization it leads to the most difficult separation of chromosomes at anaphase. It is to this that the wide-spread, probably universal, occurrence of some degree of terminalization is to be attributed, and especially its characteristic occurrence in natural ring-forming genera such as *Oenothera, Rhoeo, Anthoxanthum* and *Campanula* (*cf.* Darlington, '31b).

(iv) Polarization of the chromosomes at zygotene will make for regularity of pairing in any organism and will considerably modify the results of pairing in hybrids. Any change in the linear homology of the chromosomes will interrupt their pairing at zygotene, as may be inferred not only from direct observation but from crossing-over frequencies (Dobzhansky, '31). If pairing therefore begins at the ends, the segments whose pairing will suffer will be the proximal ones. This is particularly important in ring-forming complex-heterozygotes whose complex differences are probably localized near the middles of the chromosomes (Darlington, '31c, App. V.). It is probable therefore that *Oenothera, Rhoeo* and other genera of this kind have a polarized nucleus with a "bouquet" stage—although this has not yet been shown. It may be mentioned, by the way, that polarization limits the exchange of partners at zygotene and so probably prevents the formation of multivalents where they would otherwise be expected—as in the polysomic spermatocytes of *Camnula* (Carroll, '20; *cf.* Darlington, '31d).

(v) Crossing-over is generally restricted (in sex chromosomes and autosomes) in the heterozygous sex in animals (Haldane, '22). Since genetic factors affecting chiasma frequency and distribution seem to affect all the chromosomes alike, it seems probable that factors restricting and localizing crossing-over have been selected in the heterozygous sex. The same process must be the basis of secondary sexual differentiation, for only by the abolition of crossing-over between the original homologous sex factors and others in the sex chromosomes establishing secondary differences can these differences become permanent (Darlington, '31a). Possibly in some species the same end has been attained by the selection of factors which hasten the prophase development of the sex chromosomes differentially and thus inhibit their pairing.

(vi) Most sexually fertile species show a remarkable uniformity in the size of the chromosomes making up their complements. This can not be accounted for by the known randomness in the occurrence of changes in their structure. Rather, since the pairing of chromosomes seems to be determined by the formation of chiasmata, this uniformity is necessary for their regular pairing if the chiasmata are regularly distributed in proportion to the length of the chromosomes. When therefore great differences are found in the size of the chromosomes making up a set, this is associated with a greater frequency of chiasmata in proportion to their length in the short chromosomes (*e.g.,* in *Hyacinthus amethystinus,* unpublished). Alternatively, where this is not the case, the small chromosomes fail to pair and do not form a regular part of the complement (*e.g.,* fragments in *Tradescantia* and *Fritillaria, cf.* Darlington, '31b) or they pair momentarily at metaphase by an exceptional method, probably found only in the Hemiptera. Wide variation in chromosome size in the permanent complement is therefore conditioned by genetic properties of the organism in regard to chiasma-formation. In this regard it is interesting to

notice that in *Gryllus,* where the autosomes have undergone fragmentation, the X-chromosomes are unaffected, presumably because chiasmata are formed less frequently in them (Ohmachi, '29; *cf.* also *Orphania denticauda,* de Sinéty, '01).

(vii) *Causation of meiosis.* Meiosis must be supposed to have arisen as an aberration of mitosis. Since this must have occurred in the rather remote past it may seem absurd to speculate as to its conditions. But three considerations make it reasonable: (i) Both meiosis and mitosis preserve certain characteristics which being universal must be supposed to have been originally present. (ii) Meiosis must have arisen in one step from mitosis, for unless at the first attempt it gave an accurate reduction it would only destroy the cell-generation that led up to it. (iii) Genetic conditions have been revealed in two genera which as far as possible reconstruct the scene.

In *Matthiola,* meiosis appears to start so late that the chromosomes do not contract as much as normally, and pairing is liable to failure. In polymitotic *Zea,* mitosis appears to start too early, so that the chromosomes contract more than normally and some pairing occurs. The abnormalities both in *Matthiola* and in polymitotic *Zea* therefore indicate that meiosis is determined by a premature prophase interrupting the resting stage before the chromosomes have divided and leading to their pairing, chiasma-formation (crossing-over) and a greater contraction of the chromosomes at metaphase (*cf.* Darlington, '31b). The occurrence of meiosis at a particular stage of development we can therefore regard as determined by genetic factors and as an adaptation necessary for sexual reproduction. The evidence for this *precocity* hypothesis has been systematically examined in the contribution referred to (1931b).

(viii) The distinction between structural and genotypic effects enables us to analyze the conditions of the reverse change, by which parthenogenesis and other forms of apomixis have replaced sexual reproduction,

more exactly than has been possible hitherto. These seem to be the following:

(a) The genetic property of permitting *either* the development of the specialized female germ cell without fertilization (haploid or diploid parthenogenesis) *or* the development of unspecialized tissue into an embryo (apospory, apogamy, nucellar embryony).

(b) The property of producing, in the first type, unreduced germ cells and, in the second type, inviable germ cells. This may be either (i) genetic (as pointed out above) (ii) structural, *i.e.,* depending on the structural relationship between chromosomes of opposite parents in a hybrid (especially in the second type), or (iii) a combination of the two. The second condition probably always leads to the third and is responsible for the complexity of many cases in plants. Thus it is possible for a sexually sterile interspecific hybrid having the necessary genetic conditions to be, parthenogenetically, fairly fertile, owing to the formation of unreduced germ cells in the way shown by Rosenberg ('27). Unreduced gametes will, in the original hybrid, be formed rather irregularly, as found in *Ochna serrulata* by Chiarugi and Francini ('30). Their formation is associated as might be expected, on the precocity theory of meiosis, with a time irregularity: it anticipates normal meiosis by five days. But gametes formed in this way can show segregation on a lower scale than normal mendelian segregation, owing to the occasional pairing of chromosomes, crossing over between them and consequent segregation at the division of restitution nucleus. This will be important because the rarer the pairing the greater, we must suppose, the differences to be segregated. Segregation should therefore take place in a new parthenogenetic hybrid on a scale appropriately reduced. If the occurrence of meiosis is affected (as we know it may be) by genetic factors in regard to which segregation takes place, then secondary adaptation will occur leading to the total and regular suppression of meiosis, since segregates

having such suppression will be more fertile. Mutation found by Ostenfeld ('20 *et al.*) in parthenogenetic *Hieracia* is a proof of the occurrence of the processes I am imagining and, whether or not it has the cause I suggest, its result will be the same.

III. Conclusion

The assumption of continuity and permanence in the structure of the chromosomes, combined with a potentiality for random change, allows the consideration of chromosome form and behavior from the point of view of adaptation and evolutionary theory to only a limited extent. This is due to the fact that changes in chromosome structure and number must as a rule be vastly more important in their genetic effect on the organism than as adaptations to their mechanical function. Two notable exceptions suggest themselves. Polyploidy and ring-formation provide the means by which hybrids (of sudden and of gradual origin, respectively) may become true-breeding species (Darlington, '28, '29a). With these exceptions we may say that where structural changes are concerned, adaptation of the chromosomes to their mechanical functions is subordinate to adaptation to their physiological functions.

But the assumption of genetical control allows the theory of adaptation to be carried to its logical conclusion. Some of the examples of adaptation cited above have long been recognized and in the absence of the principle of genetic control they have been the subject of a variety of explanations. These are analogous to the explanations attempted in regard to evolution in general as alternatives to the adaptive principle which has been applied here. They may be classified (neglecting fallacies, such as telosynapsis, which are based on erroneous observation) as follows:

(i) *By teleology.* Reduction of chromosome number at meiosis and therefore the occurrence of meiosis was customarily explained by the older investigators as having the *purpose* of compensating for the addition in fer-

tilization. Similarly, it has been suggested that polyploids owe their origin to chromosomes doubling their number in order that they might have partners.

(ii) *By false analogy.* The differential condensation of the chromosomes has been regarded as a symptom of degeneration and the sex chromosomes have been supposed to undergo such changes on the analogy of the evolutionary processes which are inferred in impermanent structures. Similarly, processes of "historiation," "quantitation" and the like have been assumed in chromosomes (Delaunay, '30; Navashin, '31, *et al.*). Such descriptions skip a necessary step in the argument and imply inherent changeability. Yet the chromosomes do not change of themselves but rather through the accidents of their relationship with external mechanical forces (as the primary changes in irradiation) and with one another (as in the secondary changes in crossing-over). Such terms therefore contradict the theory of continuity without evidence, and leave us in an inconsistent position.

(iii) *By petitio principii.* Reuter ('30) has avoided both these fallacious short cuts and attempted to arrive at a purely causal explanation of chromosome behavior. This consists in the assumption of "rein bio-physikalisch-chemische Kräftespiele," which determine chromosome behavior. Then, if the proper forces are assumed (such as agglutination, affinity and the like) the observed results ensue. This method of argument makes the earlier fallacies unnecessary and brings us back to where we started. The examination of chromosome behavior by this method seems to have been entirely fruitless. The Kräftespiele remain an enigma.

Begging the question in this way is not the beginning of an explanation. We must not attempt to consider ultimate "bio-physico-chemical reactions" until we have determined immediate conditions. This is possible by genetical analysis, and the present attempt, faulty as it no doubt is, shows the only direction along which inquiries of causal relationships can be profitably pursued.

SUMMARY

It is shown that in twelve different ways the behavior of the chromosomes of an organism in the resting nucleus, in mitosis and meiosis, is subject to the control of the genotype. The chromosomes are therefore subject as a whole to the variation in their parts. This qualification of the principle of continuity, first, makes it necessary to consider all variation in chromosome behavior as the result of either genotypic or structural change and, secondly, makes it possible to examine this variation in terms of evolutionary theory, considering it in every detail as the product of an adaptive process. Such a point of view is important in considering the origin of meiosis, sexual dimorphism and parthenogenesis. Some of the genotypic differences observed raise questions of physiological interest (affecting, for example, the organization of the hereditary material in the chromosome) but these are not yet ripe for discussion (*cf.* Haldane, '32). The problem will be dealt with more fully elsewhere.

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THE NINE PRINCIPLES OF EVOLUTION REVEALED BY PALEONTOLOGY[1]

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IN honor of Darwin our first thought is that Natural Selection is the sole survivor of the age-long theories and hypotheses clustering about evolution. When we consider the youthful zoology and the infantile paleontology of Darwin's time (1809–82), our admiration for his genius and marvelous powers of generalization constantly increases. What would his generalizations have risen to with our present knowledge? The ratio of the 8,767 vertebrate species known in his time to the 65,939 species known in 1925, nearly 8 to 1, is about the measure of the biological progress of the first century of evolution. In 1831 only three species of fossil elephants were known—the Mammoth (*Elephas primigenius*), the Mastodon (*Mastodon americanus*) and the southern mammoth (*Elephas meridionalis*); now there are over 350 species and over 30 genera. Darwin foresaw the promised land of paleontology, but did not live to enter it.

It is a striking fact that the zoologists, experimentalists and geneticists who, a quarter century ago, were stoutly combatting Wallace, Weismann, and other superselectionists, have, one after another, returned to the Darwinian sheepfold and are now almost unanimously teaching their students, as if it were a demonstrated fact, that evolution progresses by the survival of fortuitously adaptive mutations. To the mind of the paleontologist these teachings are pure Darwinism camouflaged in new language. Bateson, founder of the genetic school, is the only one to confess frankly his utter failure to explain the origin of species; few have displayed similar courage.

[1] Fourth paper in the "Centenary of Evolution" Conference before the British Association, September 24, 1931. This is the sixth of a series of papers by the author on the origin of species; the seventh will be entitled "The New Concept of Evolution."

Selection alone has stood the test of survival of the fittest, yet we must severely limit the powers of selection as Darwin imagined them in his earlier and more sanguine frame of mind, and glean the elements of truth pervading all the other hypotheses and theories.

Genetics a Science of Heredity, Not of Evolution

The geneticists are trying to make evolution fit the genes rather than to make the genes fit evolution.

Far from marking any real progress toward the eternal question of the origin of coordinated mechanical adaptations, the experimental zoologist and geneticist are making little advance along the biochemical or experimental lines. In order to ascertain the state of current observation and opinion on the mutational origin of species, sub-species or adaptive mutations, as well as on the experimental results of biochemical, biophysical and endocrine action, the following questionnaire (slightly modified) was sent out on July 17, 1931, to forty-one leading zoologists:

1. Do you know of a single concrete case of the origin of a species, of a sub-species, of an adaptive mutation of De Vries, or of a single adaptive character, arising suddenly by mutation or saltation either under natural or experimental conditions?

2. Granted that permanent hereditary sports, mutations and modifications of existing characters are being produced by biochemical and biophysical means, has a single adaptive character been produced by such means through experiment in your laboratories?

3. Granted that profound changes in coloring, in form, in somatic proportion, in the developmental acceleration or retardation of characters, may be produced by glandular action, is there sufficient evidence that Nature has ever proceeded in this way except in producing immunity and non-immunity?

Up to August 13 seventeen replies had been received. To Questions 1 and 2 the prevailing answers were negative as indicated by the reply "No." More or less positive or affirmative replies were also received to Question 1, but analysis of most of these replies indicates that the crucial element in Question 1 is evaded, namely, *the ori-*

gin of adaptive characters. No doubt certain mutations do survive; many of them are recorded in the answers to the questionnaire; Crampton has discovered mutations in his monographic researches on the mollusc *Partula;* Chapman in the avian genus *Buarremon;* the short-legged Ancon sheep is a classic; certain biomechanical mutations in the pelagic Ciliata, such as the spiral shelf of *Xystonella scandens* observed by Kofoid, may be of sudden or mutational origin, although this is not proved. The answers of seventeen zoologists to Question 2, as to adaptive biochemical origins by experiment, were uniformly negative; new and hereditarily permanent mutations may be aroused by more or less violent chemical or physical means, but not a single one is known to be adaptive. The answers to Question 3 were five negative and five affirmative; the negative squarely meeting the question, the positive reaffirming the granted postulate that endocrine secretions profoundly modify all existing characters and processes of development, but not a single case can be cited wherein a new biomechanical character has arisen through endocrine action.

ZOOLOGY, COMPARATIVE ANATOMY AND EMBRYOLOGY REVEAL TEN PRINCIPLES OF EVOLUTION

Although primarily an original observer rather than a collator of other people's ideas, Darwin was more or less familiar with ten of the principles of "biomechanical" adaptation which had been observed in the hard parts of animals from the time of the earliest Greek anatomists and philosophers. Throughout his frequent discussions of the biomechanical evolution of animals are included the bony and muscular biomechanical adaptations brought about through processes of (1) degeneration, (2) development, (3) compensation, (4) economy, (5) change of proportion, (6) coadaptation, (7 and 8) acceleration or retardation, (9) self-adaptation, and (10) sports and discontinuities.

Note that all these ten pre-Darwin principles of adaptation relate not to the *origins of organs,* but to the *modification of existing organs,* as of the wings of the duck, the neck of the giraffe, the speed of the wolf. Darwin realized that the weak point in his theory was in the matter of origins; he could, and did, largely explain the survival value of organs once established, but was hard put to place a survival value on fortuitous variations.

These ten zoological principles are as follows:

ZOOLOGIC MODIFICATION OF EXISTING ORGANS, ONTOGENY

Discovered in Anatomy and Embryology.

Known to Darwin.

1. Biomechanical *onto-retrogression* (Aristotle), degeneration, atrophy of organs.
2. " " *-progression* (Aristotle), development, hypertrophy of organs.
3. " " *-compensation* (Aristotle), metatrophy and eutrophy of organs.
4. " " *-economy* (Aristotle), metatrophy and eutrophy of organs.
5. " " *-allometry* (Lamarck-Darwin), allometrons, changes of proportion in organs.
6. " *co-adaptation* (St. Hilaire), coordination, correlation of organs.
7. " *onto-acceleration* (v. Baer) into earlier growth stages of organs.
8. " " *-retardation* (v. Baer) into later growth stages of organs.
9. " *auto-adaptation* (Goethe) through principles 1–8.
10. " *onto-saltation* (St. Hilaire), sports, discontinuities.
11. *Cellular continuity of the germ plasm in the perpetuation of organs* (Weismann).

One great principle remained to be established after Darwin's time, namely, (11) Weismann's cellular continuity of the germ plasm, in antithesis to all pangenetic or somatic hypotheses of the origin of hereditary characters. To Weismann's principle of cellular continuity paleontology adds the following nine new principles of germinal evolution.

PALEONTOLOGY REVEALS NINE NEW PRINCIPLES OF GERMINAL EVOLUTION

The "biochemical" adaptations underlying coloration, as well as the "biophysical" adaptations controlling many of the animal instincts, are beyond the ken of the

paleontologist who is bounded by his biomechanical fossils. Nevertheless, paleontology is the acid test; *paleontology is evolution.*

In this struggle for existence of bygone theories and of new hypotheses, it seems that paleontology, with its world of new and wholly undreamt-of evidence as to the *origin of adaptive biomechanical characters,* serves as the two-edged sword of biology; it cuts hypotheses unfit to survive; it strengthens hypotheses fit to survive. It calls for conceptions of a new and synthetic physicochemical order to supplant outworn hypotheses dating back to Empedocles (600 B. C.). Paleontology disestablishes the entelechy hypothesis of Aristotle (300 B. C.) and of all his "vitalistic" followers like Driesch and Bergson. It substitutes for Aristotle's "internal perfecting tendency" the idea of adaptive reaction and interaction of internal with external energies which has been formulated (Osborn, 1912–1929) into a new *tetraplastic* principle of the "four inseparable energetic factors of evolution," namely: (1) physical environment, (2) ontogeny including habit, (3) living environment, the biota, (4) the germ plasm or *geneplasm.* The above energetic complex is subject to the non-energetic selection—survival of the fittest.

This tetraplastic principle which seeks to combine the elements of truth in preceding hypotheses and theories has, thus far, won no acceptance.

In causation of the origin of species and subspecies, paleontology unites with modern field zoology in firmly establishing the direct action of environment (Buffon, 1755–Wagner, 1870) on the germ plasm as a causative factor. It disestablishes the habit-inheritance law of Erasmus Darwin and of Lamarck (1790–1809); through auto-adaptation it establishes habit as a *guiding* principle in evolution, but not in the Lamarckian sense. Paleontology eliminates selection from the energetic complex; it establishes the non-energetic selection as a universal and outstanding guide and principle of progress

from the beginning of time; it disestablishes the third and fourth principles of Charles Darwin (1859), namely, of the origin of adaptations through the survival of the fortuitously adaptive. It firmly establishes the inconspicuous adaptive origins of new characters first observed by Waagen (1869), which may be distinguished as *W.* mutations when compared with the fortuitous *D.* mutations of De Vries (1911). At least from biomechanical evolution it excludes entirely the fortuitous *D.* mutations of De Vries. It helps to disestablish the "pangenesis" of Darwin and all similar theories of the somatic origin of adaptive characters; it accordingly disestablishes the "inheritance of acquired characters" and establishes the complementary principle of the "continuity of germ plasm" of Weismann (1880). It disestablishes the superselection theories of Weismann and other neo-Darwinians. In its earliest (1806), as well as its latest phases (1931), paleontology undermines the primitive idea of "created evolution"; of recent years it tends to establish the wholly different idea of "creative evolution," which may be provisionally termed *aristogenesis.* Finally, paleontology unites with systematic and experimental zoology in compelling us to concentrate research on the origin and coordination of biomechanical characters, in the lower animals and in man, as the outstanding problem of the second century of evolution.

Paleontology (1869–1931) through a succession of discoveries has revealed nine principles of *adaptive biomechanical origin in the germ plasm.* Thus, up to the present time, we have established through combined observation in zoology and paleontology no less than twenty more or less distinct but invariably cooperative principles of biomechanical adaptation.

Of the nine principles of biomechanical origin discovered in phylogeny since Darwin's "Origin of Species" (1859), the first three were observed in fossil invertebrates, namely: (12) the *D.* mutations of Waagen (1869), in which is involved (13) the "trend" or "mutations-

PALEONTOLOGIC ORIGIN OF NEW CHARACTERS, PHYLOGENY

Discovered in Paleontology.	12.	Not known to Darwin.	Biomechanical *phylo-mutation* (Waagen, 1869), orthogenesis in new characters.
	13.	"	*-trend* "mutations-richtung" (Neumayr) in new characters.
	14.	"	*-acceleration* (Hyatt, 1880) in the evolution of single characters.
	15.	"	*-retardation* (Hyatt, 1880) in the evolution of single characters.
	16.	"	*-continuity* (Osborn, 1889–1931) vs. discontinuity in single characters.
	17.	"	*-potentiality* (Osborn, 1889–1931) in the origin of new characters.
	18.	"	*-predetermination* (Osborn, 1889–1931) in the origin of new characters.
	19.	"	*-rectigradation* (Osborn, 1889–1931) in the origin of single characters.
	20.	"	*-aristogenesis* (Osborn, 1889–1931) in the rise of grouped characters.

richtung" of Neumayr (1875); (14–15) the "acceleration and retardation" of Hyatt (1880), principles which Darwin could not clearly comprehend. In the year 1889 Osborn, at the time a convinced neo-Lamarckian, began his extremely intensive observations upon the origin and development of single adaptive characters, aided by unprecedented fossil material first in the primates and then in five independent divisions of the ungulates, wherein were revealed five previously undiscovered principles of biomechanical adaptation, namely: (16) germinal continuity versus discontinuity, and *W.* mutations ascending and descending; (17) germinal potentiality, as a basis of Lankester's principle of *homogeny;* (18) germinal predetermination, as distinguished from indeterminate origins; (19) germinal rectigradations, as distinguished from fortuitous or chance origins. Finally, in the synthesis of the orthogenetic origin of new adaptive characters in all the mammals, including man, there was reached (20) the grouped principle now provisionally termed *aristogenesis* for the want of a more appropriate word to express continuously creative adaptation.

A very important distinction is observed between *rectigradations* which are predetermined, and changes in proportion, as in the elongation of the neck of the giraffe, technically known as *allometrons,* which are not prede-

termined. Rectigradations are relatively rare, while allometrons, or changes of proportion in the teeth, skull and limbs, are constantly in progress and make up a larger part of the definition of species. Similar rectigradations arise through community of descent. Similar allometrons are constantly arising in animals of dissimilar ascent.

Whereas the zoologist, comparative anatomist and geneticist, by the very nature of the evidence at their command, find difficulty in distinguishing between the accidental, fortuitous and temporary variations, fluctuations and *D.* mutations, the paleontologist is absolutely sure of his footing as soon as he is enabled to observe the ascending geological *W.* mutations of animal mechanisms, whether invertebrate or vertebrate. He advances solely by inductive means, after the manner of Darwin. If he is not sure of the adaptive trend of a certain rectigradation in its feeble, incipient stage, he may observe it a hundred thousand or a million years later as the dominant and commanding character of the entire organism.

As Weismann spoke of the immortality of the germ plasm, the paleontologist may speak of the secular immortality of thousands of characters which he is enabled to observe through the whole cycle from potentiality and predetermination in the germ plasm until, after eons of use and service, they may subside again into the mysterious germinal substance—mysterious because utterly inexplicable. The biomechanism of the titanothere and of the elephant is due to a complex of energetic factors which is entirely beyond our present comprehension.

Conclusions

The paleontologist concludes that *the origin of species,* so far as species are defined by various stages of biomechanical adaptation, has long ceased to be a problem; the manner by which subspecies, species, genera, families and orders arise through divergence, acceleration, retardation and rectigradation is also perfectly clear.

One after another the original Buffonian, Lamarckian, Darwinian, Weismannian and De Vriesian theories of causation have failed. Each, however, contains elements of truth. As to Lamarckism, we can affirm that it is the essential living principle of biochemical reaction which as a secular effect calls forth the adaptive biomechanical response, whether in ontogeny or in phylogeny. As to Darwinism, selection acts incessantly; it originates nothing, it does not control the origin of characters; it may or may not control the rate of evolution.

All that we can say at present is that Nature does not waste time or effort with chance or fortuity or experiment, but that she proceeds directly and creatively to her marvelous adaptive ends of biomechanism.

Darwin was at once naturalist, geologist, paleontologist, zoologist, botanist. To this galactic universe of talents, he who would generalize from the twenty principles of biomechanical adaptation must also be chemist, physicist and philosopher. We await the man of genius to discover the *causes of evolution* according to an entirely new concept of evolution.

While we know infinitely more about the modes of evolution than did Charles Darwin, and while we can demonstrate beyond refutation the prevailing twenty principles of biomechanical adaptation discovered in ontogeny and phylogeny, *we are more at a loss than ever before to understand the causes of evolution.*

ON THE ORIGIN OF MUTATIONS

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I

A REVIEW of the modern work in genetics shows that many mutations both in animals and in plants have been discovered. How do these mutations arise? This is the great question in genetics.

What may prove to be a great step forward in advancing our knowledge of the mechanism of Mendelian heredity was Muller's discovery that Roentgen rays can induce mutations in *Drosophila*. Subsequently, Muller's results have been confirmed by others. Moreover, it was soon learned, both for animals and for plants, that mutations can be induced not only by Roentgen rays but also by radium and by ultra-violet rays. Even more significant are the results, reported first by Goldschmidt and later by Jollos, that increase in temperature induces mutations in *Drosophila*. These experimental findings have given genetics a new lease on life.

We are justified, I think, in drawing two conclusions from this work on the induction of mutations by radiations and by heat.

In the first place, we conclude that there is no specificity in the agents which induce mutations. Roentgen, radium and ultra-violet rays and temperature are all effective though not equally so. Roentgen rays seem at present most efficacious; heat, on the other hand, judged by the failure of some workers to obtain positive results, seems least efficacious. Nevertheless, the very careful experiments made by Jollos, noted for his scrupulous methods, place the effect of temperature beyond doubt. In some respects the effect of temperature is more significant than that of the very artificial agent, Roentgen rays. The results with these rays, however, are more theatrical than those with the natural and ubiquitous agent, temperature. Even if it prove that the action of

all agents—those now known and others which may be discovered later—fundamentally affect the cell in the same way, as, for example, by causing directly or indirectly a rise in temperature, we may still argue that in the agents themselves lies no specificity.

Too often in biology we offer explanations of the responses of cells in terms of the specificity of the outside agent. Just as often, likewise, we find that the responses are due to the cells themselves which react according to their nature—that is, manifest their peculiar kinds of irritability—in a definite way, no matter what the agent eliciting the responses may be. It is thus with the unfertilized egg cell, the muscle cell or the nerve cell. All these cells attempt to respond to the experimental agent in the manner characteristic of their responses to the normal mode of stimulation. The differences found in their responses to the artificial and to the normal agents are doubtless due to the nice exactitude of the cells as reacting systems keyed with nice exactitude to the action of the normal agents and not to the crude experimental agents. On these grounds, and in the absence so far of any proof of specificity, we conclude that agents which induce mutations lack specificity. This, if it prove true, is a help, not a hindrance, to the analysis of the mechanism of Mendelian inheritance.

We may conclude, in the second place, that the mechanism for mutations which arise experimentally lies in the behavior of the chromosomes as wholes or in the behavior of units in individual chromosomes.

The chromosome theory of heredity is one of the great contributions of modern biology. A large body of cytological and experimental data indicates that the phenomena of Mendelian inheritance can be explained in terms of the behavior of the chromosomes in successive generations—that is to say, in terms of chromosomal combinations, segregations and recombinations. Furthermore, of the individual chromosomes we may state that modern work in genetics has fully sustained Roux's

('83) assumption of the serial order of the unit factors. This is likewise true of the important postulates made by Correns ('02) and by deVries ('03) concerning the unit factors in homologous chromosomes. Morgan and his school, working with *Drosophila,* have amassed a large body of data which forms the basis of the gene theory of heredity. Many other workers in many lands have been busily engaged in adding evidence in support of the chromosome theory of heredity. To-day, this theory is almost universally accepted; it is too well known to warrant here any prolonged exposition.

The cytological evidence at hand concerning the action of rays in producing mutations also indicates an effect on the chromosomes. I need merely refer to the work of Goodspeed, who has presented a cytological analysis of the behavior of the chromosomes in mutants of tobacco induced by radium and Roentgen rays. The evidence, so far meager as compared to the older cytological work supporting the chromosome theory of heredity, accumulated by other investigators falls in the same category. Hence, we may conclude that the mechanism for experimentally induced mutations lies in the chromosomes.

Is it possible, we may now ask, to explain how this chromosomal mechanism is so affected that mutations arise? In the following pages I venture to suggest an answer. This is that the behavior of the chromosomes which is responsible for mutations is not a primary one. It is itself the effect of an antecedent reaction in the cell.

II

There exists a large body of experimental work on the effects of external agents on cells. Much of it, having to do with the analysis of problems in embryology, deals with the egg cell. This work we can use as a source of evidence, since much of the support for the chromosome theory of heredity comes from the now classic cytological work—for example, the masterful contributions by Boveri on the normal egg in various stages and phases of its ontogeny.

Agents can and do affect the cytoplasm of an egg cell without manifesting any effects on its nucleus and, therefore, presumably on the chromatin thereof.

Consider, for example, the effect of fragmenting the egg. It is well known that fragments of the unfertilized eggs of many species are capable of fertilization and normal development. The presence or absence of the egg nucleus is of no consequence to development: nucleated and non-nucleated fragments develop equally well. Such an experiment, moreover, succeeds on an egg (*cf. Chaetopterus*) whose germinal vesicle has broken down and whose first maturation spindle is forming or formed. It is likewise successful on an echinid egg which having completed both maturations contains a nucleus in the so-called resting stage.

Thus the condition of the chromatin, whether on the spindle or in the intact nucleus, is without significance for the success of the experiment. It does not succeed on an egg (like that of *Asterias* or of *Cerebratulus*) in the germinal vesicle stage. In such it is necessary to await the dissolution of the germinal vesicle and the consequent appearance of the spindle; fragments now made are readily fertilizable. This indicates that a substance (or substances) diffusing into the cytoplasm with the breakdown of the germinal vesicle is necessary for the fertilization-reaction between egg-plasma and sperm; it does not mean that the chromosomes *per se* as discrete bodies affect the results of inseminating the fragments. From another angle, however, the diffusing stuffs from the germinal vesicle may be important: they may be the *modus operandi* of the exchange between chromatin and cytoplasm, thus accounting for differentiation in the cytoplasm and the impress of the chromosomal garniture on the plasma. In a similar way, it may be that with each nuclear breakdown at each stage in the whole ontogenetic process, substances metabolized by the chromosomes pass out into the plasma to order the development and to fix the genetic constitution of the embryo. But this is speculative.

Fragmentation of an egg with intact ovotid nucleus, even when most carefully done—as for example, by Tennent, Taylor and Whitaker—certainly disrupts the original organization of the intact egg. The normal nucleo-plasma relationship is upset in the fertilized nucleated fragments in which the ratio of chromatin to plasma is greater. In the larger of the fertilized non-nucleated fragments the ratio is less, because they of course contain only paternal chromatin. And yet there is no evidence that fragmentation, by careful cutting, affects the egg nucleus; chromatin does not escape. True, there is the remote possibility that fragments obtained by shaking an unfertilized egg with an intact mature nucleus might contain scattered chromatin, as Boveri in a posthumous paper felt impelled by rigorous self-criticism to suggest as a possible interpretation for one of his experiments. We should recall, however, that vigorous shaking, like strong centrifugal force on some eggs (*cf.* Conklin's work on ascidian eggs), may alter reactions in egg plasma as revealed by the egg's subsequent development. This has been clearly shown—by Boveri, Mrs. Theodor Boveri and others—especially for echinid eggs shaken soon after insemination. We may conclude that fragmentation by careful methods does not affect the egg nucleus or the chromatin thereof.

The effects on marine eggs of changes in temperature, in salinity or in hydrogen-ion concentration vary, depending on the eggs. For a given egg these effects vary before and after insemination. For the fertilized egg again there is a differential effect of the agent which runs with the mitotic cycle: the response of the fertilized egg to changes in its environment varies with stages of mitosis. But there is no evidence to indicate that this effect is primarily or alone on the nucleus or its chromosomes. On the other hand, there is evidence that the effect is primarily on the cytoplasm.

Now these changes in temperature, in salinity and in hydrogen-ion concentration of the sea-water may visibly affect the cytoplasm without demonstrable effects on the

nucleus. Where they do affect the nucleus, they do so after changes have taken place in the cytoplasm. Always, therefore, the cytoplasmic changes come first. Indeed, within optimum range, the magnitude of the cytoplasmic changes determines that of the nuclear. There is also a relationship between the duration of the exposure of the cytoplasm to these changed environmental factors and the quality and character of the cytoplasmic reaction. Again the intensity, that is, the strength of the change in the environment, determines the rate of the cytoplasmic response; and within optimum range, this rate determines the degree of the nuclear response.

Specific examples to support these statements, one may find in the literature on fertilization, experimental parthenogenesis, experiments on cell division and on development. Always changes—in some eggs most profound—take place in the cytoplasm subjected to those modifications in the surrounding sea-water named above. And always these changes precede any nuclear changes. In fertilization and in experimental parthenogenesis the response of the cytoplasm to these outside agents is most marked.

If the reader wish, he may consider this argument wholly lacking in force. On grounds of logic, then, I take the position that if he contend that these changes in the external medium affect the nucleus primarily and that the cytoplasmic response is a consequence of the nuclear, the burden of proof rests with him. Certainly, I may at least say that cytoplasmic response precedes the nuclear.

For many cells it is true that the display of mitotic activity visibly depends upon the condition of the cytoplasm. A certain complex of physical and chemical conditions in the cytoplasm is necessary for the initiation and completion of mitosis. Conklin has described the behavior of the spindle in the egg of *Sycotypus*. Here the spindle lies in very fluid cytoplasm and grows at the expense of the loss of this fluidity. Reaching a certain size, it can complete mitosis. Wilson has detailed the process of cell division in the egg of *Renilla,* in which

two nuclear divisions ensue before division of the cell body—a phenomenon deserving more study.

Egg cells have been subjected not only to changes in the temperature, in the salinity and in the hydrogen-ion concentration of the surrounding sea-water; they have also been exposed to radium, Roentgen and ultra-violet rays. One noticeable effect of such exposures is on the cytoplasm. Work on the egg of *Nereis* may be cited.

Some years ago Packard reported results of exposing eggs of *Nereis* to radium. The first visible effect is cytoplasmic. In consequence or at least in sequence to this follow modifications in nuclear behavior. Redfield especially has analyzed the cytoplasmic response displayed by this egg after exposure to various rays. Also in this same egg Just found that ultra-violet rays induce profound cytoplasmic changes which make possible abnormal nuclear behavior.

It is well known that radiations, especially radium and Roentgen rays, are most effective on cells after nuclear breakdown. An exposure to these rays which does not affect the cell while its nucleus is intact has profound effect when after breakdown of the nucleus the cell is in various stages of mitosis. It is therefore held that such radiations directly affect the chromosomes. Indeed, some workers consider—I think it is no exaggeration to say—that Roentgen rays, for example, are wholly a chromosome-affecting instrument. A moment's reflection, it seems to me, gives rise to some questioning of this position.

In the first place, radiations are not alone among agents which are more effective on cells in stages of mitosis than on those with the so-called resting nuclei. Certainly, for egg cells there is abundant evidence which indicates that paralleling the rhythm of mitosis is a rhythm of susceptibility and resistance of the cytoplasm to many and diverse experimental agents. The rhythm of nuclear division is itself parallel with the rhythm of physico-chemical and morphological changes in the cyto-

plasm. If, therefore, we argue that Roentgen or radium rays are exclusively chromosome-affecting agents, because of their greater effect during mitosis, we must then argue similarly concerning the effect of all external agents which behave likewise. It is simpler to place the action of radiation in the same class with these other experimental agents.

What is true is rather this: radiations are far nicer agents than, for example, hypo- and hyper-tonicity. Their effects are more rapid, more exact and more widespread in a given population of cells. This, in my judgment, is the chief value of radiations as experimental agents—not their so-called specific action on chromatin.

Secondly, ultra-violet rays which have feeble penetrating power act in some cases as do Roentgen or radium rays; ultra-violet then affects cells more superficially. Hence, it is not the penetrating power of the rays and so their power to reach the more deeply lying chromosomes which is responsible for their effects. Moreover, we must know for deeply penetrating rays that they produce no effects on the cytoplasm in the course of their penetration to reach the chromosomes.

Nor does the fact that in Roentgen therapy there is a difference in the susceptibility of human cells vitiate the argument. We still need to know on what this difference depends. And if we agree that this difference depends on the mitotic capacity of the cells—cells highly endowed with division and growth capacity being more susceptible —still are we far from proving that the rays are chromosome specific in their action. There is still the possibility that such division- and growth- capable cells have cytoplasms whose condition renders them more susceptible than other cells to radiation.

This consideration leads us then to the third point: namely, that materials entering the cell—gases, water, etc.—come into relationship first not with the nucleus but with the cytoplasm. We may here reason, therefore, from analogy. Consider oxygen consumption.

There was a time when biologists assumed that the nucleus is the seat of cellular oxidations. The presence of iron in the nucleus was postulated as part of the oxidation mechanism. On *a priori* grounds one would assume that oxygen entering the cell would combine with cellular constituents lying in the cytoplasm between the cell boundary and the nucleus. Now we know that this is at least nearer the truth: oxygen consumption is a function of cytoplasmic structure. The beautiful work of Warburg, in the forefront of modern physiological research, puts this beyond doubt. Moreover, Lynch was unable to demonstrate the presence of iron in the nucleus of sperm, though his method was most excellent.

What is true of oxygen is doubtless true of other substances that enter cells: they affect the cytoplasm first. In the case of agents like the radiations under discussion, which possess destructive action to a high degree we must prove that they can penetrate cells without influence on the cytoplasm lying in the path which they traverse before reaching the nucleus. It is more reasonable to assume that radiations do affect the cytoplasm.

All these considerations the reader may deem as of no great weight. They constitute in the whole merely a suggestion. No biologist to-day assumes that the nucleus can play a rôle in vital phenomena apart from the cytoplasm; both constituents of the protoplast are necessary. On the other hand, the conception of the cytoplasm as "the kitchen of the nucleus" is fraught with consequences ill to the resolution of problems of the biology of the cell. However, for the sake of analysis, though we firmly believe in the subsidiary rôle of the cytoplasm, it would seem worth while to examine this point of view: namely, that nuclear (and therefore chromosomal) behavior is secondary to that of the cytoplasm. I suggest that the cytoplasm determines nuclear behavior; the chromosomal behavior then is an expression of a more fundamental cytoplasmic activity.

Experimental cytology gives instances of aberrant behavior of the chromosomes in egg cells. This work indi-

cates that the experimental agent affects the cortical cytoplasm. The theory advanced here is that this action of the agent on the egg cortex is responsible for secondary effects on the whole egg cell. Among such effects is the aberrant behavior of the chromosomes.

III

In the early days of the modern work on genetics, the experimental analysis could not have got very far without the cytological work on the behavior of the chromosomes. In the first line of this classic period for cytology stands Boveri's work. Consider, for example, his beautiful studies on multipolar mitoses in echinid eggs.

Boveri's masterful analysis of these experiments on dispermic fertilization excites only admiration for his genius. Development depends, this work established, not on the number but on the proper combination of chromosomes. Here is a possible starting-point from which we may begin an attempt at the union, nowadays seemingly hopeless, of genetics and the physiology of development. But it is in another direction that this work of Boveri's demands attention.

A fundamental postulate of genetics is that the chromosomes of a given species differ qualitatively. Boveri's work on dispermic echinid eggs was therefore important, since it proved these qualitative differences. Normal development, he showed, depends upon the proper distribution of chromosomes to the cells derived by cleavage from the egg fertilized by two spermatozoa and therefore possessing an extra set of paternal chromosomes.

To secure dispermic or polyspermic fertilization of an echinid egg, one must first weaken it or employ heavy insemination. Heavily inseminated eggs do not all fertilize. In some cases, such eggs never fertilize. Since, however, after weakening the egg, polyspermy is rendered more easily possible, it may be assumed that where such weakening has not taken place those eggs which are fertilized by more than one sperm are weakened at the outset. Or, we may assume, that heavy insemination

itself induces or hastens a lowered resistance to supernumerary sperm on the part of some eggs among a population preponderantly monospermic. Certainly, experimental polyspermy more easily takes place in weakened eggs which show a slower response to insemination. In normally polyspermic eggs it is doubtless true that the slow reaction of the egg to insemination renders possible the entrance of supernumerary spermatozoa, but in these eggs only one spermatozoon unites with the egg nucleus; development always proceeds with the diploid number of chromosomes.

The aberrant development of the blastomeres in Boveri's experiments is due to the wrong combinations of chromosomes. But these combinations themselves depend upon the weakened conditions in the cytoplasm which make dispermy possible. In the normal egg the unimpaired cytoplasm protects against disorder of the chromosomes.

The most important work on the aberrant behavior of paternal chromosomes in cross-fertilized echinid eggs is that done by Baltzer ('70, '10). In certain crosses the paternal chromosomes fail to take part in the mitotic process. Instead they are eliminated from the spindle. Other workers have made similar observations. The possibility for cross-fertilization in all these cases is rendered greater by injuring the cytoplasm. Here again, therefore, the condition of the cytoplasm determines the behavior of the chromosomes. To induce cross-fertilization especially between widely separated species, for most eggs at least, one must impair the integrity of the eggs' ectoplasm or cortex. Such an impairment means a weakened cytoplasm. Like experimental polyspermy, cross-fertilization succeeds best after injuring the cortex of the egg.

A point here must be emphasized. Too frequently biologists speak of the incompatibility of chromosomes to account for their behavior in cross-fertilized eggs. Thus, the elimination of foreign chromosomes is held as

evidence for this. As a matter of fact, chromatin may be eliminated in *straight* fertilized eggs, as Gray has shown. Here there can be no question of the incompatibility of chromosomes in foreign cytoplasm. Rather, the chromosomes fail to take part in the ensuing mitoses because of the weakened condition of the cytoplasm previously treated—in Gray's work by hypertonic sea-water. Hertwig's work on the effect of temperature on the sperm nucleus in the echinid egg and Wilson's with ether are amenable to the same interpretation. Moreover, it could scarcely be argued that the specific sperm chromatin fails to play a part in the fertilized egg of *Rhabditis aberrans* because of its incompatibility! I have found in straight fertilized eggs of *Echinarachnius* that the *whole* egg nucleus may fail to take part in the cleavage mitosis. Such eggs are injured. The behavior of monaster eggs (M. Boveri, Th. Boveri) is a case in point. The abnormal behavior of chromosomes in these cases is clearly due to injury of the superficial cytoplasm brought about by vigorous shaking at a time after insemination when the cortex is most susceptible to experimental treatment.

The effect of radiations (referred to in the foregoing section) on straight fertilized eggs may be recalled. Here again the aberrant behavior of the chromosomes is in consequence of cytoplasmic injury or change. This injury is a definite cortical injury, according to Packard, Redfield and Just.

In all the foregoing it is safe to conclude that the injury to the cytoplasm is a cortical injury. We may therefore make the hypothesis that in cortical behavior lies the cause of the behavior of the chromosomes. Normal chromosome distribution and combinations depend upon the integrity of the cortex; their aberrant behavior is the effect of the loss of this integrity. Such behavior may manifest itself in chromosomal elimination, fragmentation, and the like.

Further, arguing from the foregoing, we may assume that the orderly normal behavior of the chromosomes depends upon cortical condition. This would mean, therefore, that chromosome-behavior is not a primary one, but rather that it is an expression of the ectoplasmic reactions.

In a sense, the normal behavior of the chromosomes is too rigidly mechanical for them to be responsible as primary agents in heredity. Such a mechanism strongly suggests some deep-seated force of which they are the expression. If we compare them to soldiers going through maneuvers, we must then assume some source of command. Their orderly behavior is not automatic but conditioned. It is as though we were to look on puppets in a show—however exact and wonderful their movements, however much we forget at times that they are puppets, soon we recall them for what they are. The chromosomes in this sense mark the reactions of the cytoplasm in which they lie. That these reactions are so far too subtle for analysis should not down us; we must not in our enamored state over chromosome behavior become blind to an alternative hypothesis.

From this point of view the gene theory is a conception too ultra-mechanistic to yield further profitable results. A conception in terms of reaction-velocity *in the cytoplasm,* difficult though it may be for attack, must be considered. And in time doubtless we can discover a mode of attack.

For me it is difficult to divorce nucleus (and chromosomes) from plasma. Nor can I conceive that even the most ardent supporter of the gene theory does so. The cell is a unit: the nucleus influences the plasma, the plasma the nucleus. The cell reacts as a whole. Sharply to divorce these two constituents of the protoplast is to make them abstractions. Furthermore, we can not isolate cells from time and space, as we all realize. If we could more properly evaluate the time-order in cell-processes, their reactions, their antecedent behavior in terms

of cause and effect, we could go farther. The analysis of the time-order in cortical reactions and response is possible. A great deal could be done by a more careful study of cortical structure in relating its changes in time.

So with space. One can not overlook the environment of cells. This is most apt here. At the outset, the development of the modern school of genetics began with the analysis of sex with special reference to its relation to the presence of a particular chromosome or chromosomes. Now surely we do not hold to the early and rigid theory of sex—especially in the light of the work by Hartmann, Herbst and others.

It is highly probable that the first effects of the environment manifest themselves on the cortex. It stands as the medium of exchange between the cell's inner and outer worlds. It is first impressed. The superficial reactions in protoplasm therefore come first. And these reactions certainly must affect the whole cell-system.

In time doubtless our biology of the cell will in all its manifold subdivisions reach the point of excellence attained by Warburg's work. Some method of attack will be devised for the analysis of the fundamental problem in cytology. This will doubtless be physiological. In the meantime, we must appreciate fully the morphological substratum of cells. Hence, this present attempt.

To many readers this discussion doubtless will appear wholly illusory and fantastic. I own that it is speculative. But I offer it as a suggestion—not at all as an antidote for the gospel of the original and ultimate dominance of the gene as the forerunner of all cellular metabolism.

We can not always go forward by blind acceptance—so dogma arises. Science does not progress thus. The present state of biological thought and work augurs some kind of generalization. In terms of structure, in terms of reactions both within and beyond its boundaries, the cortical protoplasm appears important for vital phenomena.

DIPLOID MOSAICS IN HABROBRACON

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Two kinds of mosaics reported as occasionally found in Habrobracon are haploid mosaic males from heterozygous virgin mothers and gynandromorphs or sex mosaics from mated females which may be homozygous or heterozygous. The frequency of discovery of the former has been variously estimated as one in 500 or one in 5,000 male offspring from heterozygous females, depending upon stocks used and conspicuousness of the character difference in the segregating fraternities. Gynandromorphs are about equally frequent in relation to females. Genetic evidence indicates that both of these types of mosaics develop from binucleate eggs and that in the case of gynandromorphs one of the nuclei is fertilized.

Egg binuclearity has been postulated to account for a minority of the mosaics found in Drosophila, while the great majority are accounted for by chromosome elimination in an early embryonic stage. The reverse condition obtains in Habrobracon. There have been previously reported three diploid mosaics. One female (279) from a cross of female with the recessives, ivory eyes and reduced wings, by type male, was type except that the right primary wing was reduced. It bred as a heterozygote of normal fertility producing type, ivory, reduced and ivory reduced. One female (313) from orange-eyed female by ivory reduced male was orange as expected, except that the left primary wing was reduced. It bred as an orange-ivory compound heterozygous for reduced producing orange, ivory, orange reduced and ivory reduced. One biparental male (318) from ivory reduced female by ivory male was ivory as expected, except that the left wings were both reduced. It proved completely

sterile, although matings were observed with six females which produced males only, 946.

Four new diploid mosaics have been found.[1] A type female heterozygous for cantaloup (eye color), c, for long (antennae and wings), l, and for semilong (antennae and wings), sl, was crossed with a cantaloup long male. Cantaloup and long are linked with about 10 per cent. crossing-over, while semilong segregates independently. The cross may then be expressed *Cc.Ll. Slsl* × *c.l. Sl*. Besides the expected classes of offspring there appeared a mosaic female (469).

Left antenna was long; right type. Left eye was cantaloup, right was cantaloup dorsally and black ventrally. Ocelli were cantaloup. Left wings were long, right were type. There was no asymmetry in body pigmentation. Breeding test showed that the mosaic had not mated, for males only were produced—type 4, semilong 12, cantaloup long or cantaloup long semilong 8. Long can not with certainty be separated from long semilong, since they both have curved wings. Linkage is shown by the fact that males with cantaloup eyes are long, while those with black eyes are non-long.

The mosaic may be regarded as having received *C.L. sl* from its mother and *c.l. Sl* from its father. This instance is of especial interest because two linked recessive traits appear in the same diploid mosaic. The maternally derived chromosome bearing dominant allelomorphs to cantaloup and to long was lost in early development.

Crosses were made by Magnhild M. Torivk of females with ivory eyes, o^i, and defective R_4 wing veins, d, by males with yellow (basal segments) antennae, Y. These factors show no linkage. From one of these crosses a mosaic biparental male appeared (344). Antennae were both yellow at base but somewhat darkened, as is char-

[1] The mutant traits, cantaloup, semi-long, yellow and miniature are x-ray mutations. The work reported in this paper was supported in part by a grant from the Committee on Effects of Radiation on Living Organisms, National Research Council.

acteristic of the heterozygote. There were 23 segments in each antenna. Left eye was ivory, right was ivory dorsally, black ventrally. Ocelli contained red pigment granules (modified ivory). Wings were type and symmetrical. Body pigment was symmetrical. The mosaic was tested by observed matings with four females but was completely sterile.

This specimen may be regarded as of composition $\frac{o^{i}}{O}\frac{d}{D}\frac{y}{Y}$. Dominant allelomorph to ivory was lost in early development affecting the eyes in part.

Crosses were made by Edward J. Wenstrup of females with orange eyes, o, and defective R_4 wing veins, d, (stock 3) by type males. Besides the expected classes, orange defective males, type females and type biparental males, there was found a mosaic biparental male (411). Each antenna had 22 segments. Eyes were black except for orange region in posterior portion of left. Median and right ocelli were black. Left was light but had some light-brown pigment (modified orange?). Wings were type, but left primary was shorter than right. Body pigment was lighter on left side of occiput and on right side of mesonotum. No breeding test was made.

This biparental male may be regarded as of genetic constitution $\frac{o}{O}\frac{d}{D}$ in which chromosome carrying dominant allelomorph to orange was lost from embryonic region, including part of left eye and left ocellus. Factors affecting body pigment and wing length may have been lost with the same chromosome producing asymmetry in these traits.

From a cross of ivory, o^{i}, defective, d, (stock 17) female by orange, o, miniature, m, male, there appeared a biparental male mosaic (337). Left antenna had 22 segments, right had 21. Eyes and ocelli were orange, the latter with much red pigment. Wings and legs were all miniature on the left side; all type on the right.

Venation was non-defective. Left mesonotal pigment mark was smaller than right. No breeding test was made.

Orange and miniature are linked with about 10 per cent. crossing-over. Defective is independent. The mosaic may be regarded as $\frac{o^1}{o}\frac{M}{m}\frac{d}{D}$ in which chromosome bearing M and some factor for body pigment was lost from left side of thorax.

DISCUSSION

RELATIVE FREQUENCY OR DIPLOID MOSAICS

Diploid mosaics in Habrobracon are evidently rare. The seven instances here reported stand in contrast to the 70 cases of haploid mosaic males from heterozygous virgin mothers and the 78 gynandromorphs or haplo-diplo-mosaics from mated females.

The conclusion should not be drawn, however, that egg binuclearity is about 21 times as frequent as somatic mitotic irregularity. Probably the former comes to light much more frequently than the latter. Binucleate eggs have a chance of being identified if fertilized or if they are produced by heterozygous mothers and undergo post-reduction. These facts eliminate the chance of finding them among the large numbers of haploid males in the studies on male biparentalism, for in this case mothers are homozygous. They should, however, be found as gynandromorphs among the females in these counts, and as mosaic males in the extensive counts from heterozygous mothers in linkage studies.

Somatic mitotic irregularity would probably fail to appear among the wasps developing from unfertilized eggs as in this case loss of a chromosome would be lethal. This eliminates the possibility of finding such irregularity among all haploid males.

The question may then be asked: Why do we not have as many mosaic females as gynandromorphs? A partial answer to this is to be found in the fact that gynandro-

morphs may on the average be more easily recognized than mosaic females. Contrast in secondary sexual traits is very striking and pertains to diverse parts of the body. Many of the mutant traits, on the other hand, affect only a limited region, such as eyes, and these are the very character differences used in the majority of crosses. Females may then be mosaic genetically, without having the difference show at all.

If we may judge from the seven diploid mosaics found, we may expect much more chromosome irregularity in biparental males than in females. Numbers of the former counted are very infrequent in comparison with the latter. Many crosses produce no biparental males at all, and in those that do the frequency is very low rarely rising above 10 per cent. of that of females. Nevertheless, we have among the seven diploid mosaics four males and three females. A somewhat larger number of mosaic females may have occurred than these figures indicate, inasmuch as biparental males may be more carefully observed than females, but this fact can not greatly affect the frequency, since the characters observed—eye color, wing size, body proportion—are very conspicuous.

It is to be presumed that irregularity in distribution of the sex-chromosome (?) is the cause of a biparental being a male. The cause of this irregularity may then affect other chromosomes as well, producing a greater tendency for mosaicism among such biparentals as are males.

Diploids or Haplo-diploids?

The question may be considered, "Are these mosaics completely diploid or are they haplo-diploids, gynandromorphs?"

The first answer to this is that none of them shows any trace of sex-mosaicism. Such would be expected to appear inasmuch as regions affected are extensive.

The three males given breeding test (318, 344, 469) proved completely sterile. Sterility is characteristic of biparental males whereas the only two gynandromorphs

which could be induced to mate were highly fertile. (P. W. Whiting and Edward J. Wenstrup.)

With only two exceptions (P. W. Whiting) gynandromorphs have proved matroclinous in male parts, whenever source of these parts could be identified (42 cases). In these two exceptional instances, the paternal trait was dominant. Three (313, 337, 469) of these seven mosaics showed recessives derived from fathers. Such would not occur without androgenesis involving origin of male parts from supernumerary sperm nucleus or unless trait were sex-linked and gynandromorph were formed, as in Drosophila, by loss of maternal X-chromosome. An excess of recessive traits from maternal over paternal source might have been expected, since many more crosses are made with recessive females (studies of male biparentalism) than the reciprocal.

Asymmetry in Wild Type Characters

Despite great variability, both genetic and environmental, in such traits as number of antennal segments, general body pigmentation and wing size, these traits are symmetrically developed in almost all wasps. It has been noted that haploid male mosaics and gynandromorphs show much asymmetry in these variable wild-type characters. If we except those instances in which structure was affected directly by the mutant factor so that one side of body was type while opposite was mutant and also those instances in which character was not specifically recorded, we nevertheless have a striking record of asymmetry in type traits. Of three records of antennal segment count (337, 344, 411) one (337) proved asymmetrical. Of four records of body pigment (337, 344, 411, 469) two (411, 337) proved asymmetrical. Of two records of wing size, (344, 411), one (411) proved asymmetrical. Observations were in these cases made without exact measurements, except that in case of an-

tennae, segments were counted. Such records indicate therefore striking asymmetry.

It is to be expected that chromosome elimination, just as egg binuclearity with post reduction would cause asymmetry in such type differences as are determined by multiple factors.

Correlative Gene Effect

Data are being accumulated indicating that, while in the majority of instances traits in mosaics are locally determined, there are many instances of correlative gene effect so that tissues of one genetic constitution are modified in the mosaic in the direction of the alternative trait. Ocelli of mosaics frequently show more or less of this intermediate condition. In the case of these diploid mosaics the same condition obtains. Biparental male, 344, presumably of genetic constitution, Oo^1, showed black and ivory in compound eyes, the latter little if any modified. Ocelli were, however, orange, showing distinct red pigment granules. Biparental male, 411, presumably of genetic constitution, Oo, had mixed ocelli with the lighter showing not orange but light brown pigment.

Several of these cases of "modification" may be explained by mixture of tissues of diverse genetic constitution. The matter is reserved for discussion in connection with the more extensive data in other mosaics.

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SHORTER ARTICLES AND DISCUSSION

GREEN'S STUDIES OF LINKAGE IN SIZE INHERITANCE

DR. C. V. GREEN has published in a recent number of this journal (Nov.–Dec., 1931) an important paper dealing with the subject of size inheritance in mammals. His observations were made on a cross between two species or mice which differ in body size but produce fertile hybrids. The larger parent was a laboratory race of the house mouse, *Mus musculus;* the smaller parent was a race of *Mus bactrianus* from China. The large parent was homozygous for three recessive color mutations, non-agouti (a), brown (b) and dilution (d). The small parent bore corresponding dominant allelomorphs, agouti (A^w), black (B), and intensity (D).

The F_1 animals were heterozygous for all three color genes and were back-crossed with the large triple recessive race for the purpose of detecting possible linkage between one or more of the color genes and the larger size of the recessive parent. Such evidence was obtained. Dr. Green's paper shows careful observation and conservative deduction of conclusions. I am fully convinced of his accuracy in both regards, so far as the hypothesis which he set out to test is concerned. But there are certain considerations which seem not to have occurred to him that conceivably qualify those conclusions. These I wish briefly to present.

Green's observations show unmistakable association of larger body size and brown coat color in the back-cross generation. This is shown in the significantly greater adult body weight of both sexes and in the several bone measurements and body length, which are in general greater. To some extent but less emphatically, the dilute individuals are larger than the intense ones. Green does not claim (in this paper, though he did in an earlier one) that larger size is a characteristic of the groups of individuals which have the third recessive character introduced from the large race, namely non-agouti (a). His tables show the adult body weight to be greater in both sexes in the non-agouti animals, but the bone measurements favor the agouti animals. The evidence is thus conflicting.

I am inclined to attach greater importance to the body weights than to any other observations made by Green because they alone

involve the entire organism and show its character at various stages of growth from birth to maturity. To be sure, bone measurements are closely correlated with general body weight, as I have shown in the case of rabbits, where the racial size differences studied were much greater than those involved in Green's mice. Green also finds a fairly high correlation between certain of the bone measurements but does not report on the correlations of body weight with bone measurements.

Green says (p. 504) "In the matter of weight, brown mice of both sexes are significantly heavier than blacks at the age of 181 days. A perusal of Table I, however, shows that this condition does not prevail at all ages, since in early life the situation is reversed, perhaps because of the initial effects of the dominant gene. In neither of the other factor pairs is there a significant difference in adult weight."

Nevertheless Green's Table I shows that in the case of the other two factor pairs recessives have a greater average adult weight than dominants in all cases except that of the dilute males which are less heavy at all ages, though their bone measurements as adults are greater than those of the corresponding dominants.

The greater *early* body weight of the dominant group is in contrast to the greater *adult* body weight of the corresponding recessive group not only in the case of the black gene but also in that of the agouti gene and of the intensity gene, except in the case of the male dilutes. I should be inclined to ascribe the greater early body size of the dominant groups not to "the initial effects of the dominant gene" but to heterosis. Each of these back-cross dominant groups is *heterozygous*, whereas the corresponding recessive group is homozygous for the gene under consideration. The heterozygous state makes for early accelerated growth, but does not necessarily affect the adult weight at all. This is made very clear in Waters' (1931) recent study of a Brahma-Leghorn poultry cross.

In adult weight Brahmas are large, Leghorns small and F_1 hybrids intermediate, but as chicks the F_1 birds are heavier than either parent breed, an obvious heterosis effect. A similar relation is seen in the relative weights of chick embryos prior to hatching, according to the observations of Byerly on a cross of White Leghorn fowl with Rhode Island Reds. See Castle and Gregory, 1931.

Also in crosses of rabbits, the F_1 hybrids are nearly or quite as large as the uncrossed large race in early life, but become intermediate or even closer to the small than to the large race, when adult. This is not due to the "initial effect of the dominant genes" because all the four dominant genes were present in the large race in the rabbit cross studied, yet rabbits *heterozygous* for these genes showed accelerated early growth but intermediate adult size.

The same types of growth curves are found in respect to all three pairs of genes among Green's back-cross mice. An accelerated early growth occurs in the heterozygous group (agoutis of both sexes, blacks of both sexes, and intense pigmented mice of the female sex) but the adult weight in all these groups is less than that of the corresponding homozygous recessive group. Consequently the growth curves of the heterozygotes and of the homozygotes of each factor pair cross, in the case of the agouti gene subsequent to the age of 61 days, in the case of the black gene subsequent to the age of 31 days, and in the case of the intensity gene for females only between the ages of one and eleven days. It seems fair to assume that a similar phenomenon occurs in each case, an accelerated early growth of the heterozygote class, which nevertheless is genetically the group of smaller body size as shown by its adult weight.

If we adopt this interpretation, then we shall accept Green's conclusion that there is genetic linkage between large body size and the recessive genes brown and dilution, and we shall extend the conclusion to take in the recessive gene non-agouti also. All three recessive genes were introduced into the cross in association with large body size. They also emerge in the back-cross in the same association. Does this mean that there are three separate genes (or groups of genes) for large body size located in the three chromosomes which bear respectively the a, b, and i genes? Not necessarily. I certainly should have so interpreted it, had I found such association to occur in the case of the four color genes involved in the rabbit size cross which I studied, but no evidence of it could there be found. Wherein then do the two cases differ? The mouse cross is not a cross of breeds, as was that of the rabbits, but a species cross. Gates (1926) has studied a cross of domesticated strains of these same two species using the Japanese waltzing mouse as a representative of *Mus bactrianus* (*Wagneri*). In a cross between pink-eyed dilute brown

house mice (related to the dilute brown race used by Green, both being derivatives of Little's dilute browns) and black-and-white Japanese waltzing mice, five independent genes were involved. The house mouse parent furnished three of the five recessives, namely pink-eye, brown and dilution, including two of the three recessives used by Green; the Japanese parent furnished the other two recessives, namely piebald and waltzing. In the F_2 and back-cross generations these five genes were found to keep together in their original associations in excess of the proportions demanded by random assortment, the deviation from expectation ranging from 0.1 to 8.2 times that due to chance.

In harmony with the observations of Gates, we should expect that in the cross studied by Green the recessive genes, a, b, and d, would be found together in excess of their chance frequencies. It seems clear from Green's Table I that a slightly larger body weight is also associated with this same combination of recessive genes. This does not necessitate the conclusion that larger body size is determined by genes located in the same chromosomes as a, b, and d, but only that *whatever* causes a, b, and d to go together, causes larger body size to go with them. That something is not ordinary genetic linkage (location in the same chromosome) as proved by the observations of Gates. It must be something connected with all three chromosomes and also a factor in size determination. What known structure has all these relations? Only the egg and sperm plasma which are extra-chromosomal constituents of the germ-cells. I have been inclined to look in that direction for the genetic basis of adult body size by the results of studies on rabbit crosses and rabbit embryology reported elsewhere, but I have hesitated to put forward an hypothesis so at variance with the currently accepted theory of the gene. Green's results distinctly favor such an interpretation rather than that of ordinary chromosomal linkage.

Gregory and I have shown that differences in general body size in rabbits are determined primarily by differences in rate of development of the fertilized egg. Though the eggs of different races are at fertilization indistinguishable in size and appearance, those of a large race segment more rapidly and subsequently increase in bulk faster than eggs of a small race. Differentiation however occurs at substantially the same rate in both. Consequently, at birth, large race rabbits are larger and they continue to grow faster after birth than do rabbits of a small race.

This difference in rate of development can not be referred to the action of genes lying in a single chromosome, as is shown by the manner of its inheritance, which is blending and requires a multiple factor scheme, if explained on the basis of chromosomal genes. I have been unable to find evidence of the existence of such genes in four of the twenty-two chromosome pairs of the rabbit, though I have made a search for it by the method employed by Green. I suspect that the difference in our results is due to the difference in our materials. The apparent linkage demonstrated by Green, I am inclined to think, is due to the same peculiarity of his material, which in Gates' experiments caused genes known to be located in five different chromosome pairs to segregates oftener than in their random proportions in the same association which they had in the parent species.

That chromosomal genes can influence the size or form of particular parts of the organism has been amply demonstrated. I am quite willing to concede all that Lindstrom claims in this regard. He has demonstrated the existence of linkage between genes governing the size and color respectively of the seeds of maize and of the fruits of the tomato. Among animals a case equally clear was demonstrated by Gates, the linkage between short ear and dilute pigmentation in mice. The entire short-ear effect is here determined by a single gene. The case is very different in the inheritance of ear-length in rabbits. Here the ear-length is a function of general body size, with which it is closely correlated, and it is doubtless determined by the same causes. The same developmental rate which produces a large rabbit will produce a long-eared one, and the developmental rate which produces a small rabbit will produce a short-eared one. What we must look for in this case is a genetic basis for rate of development. Such a basis in my opinion is more likely to be found in extra-chromosomal components of the germ-cell, since linkage can not as yet be demonstrated with chromosomal genes.

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GENETIC LINKAGE IN SIZE INHERITANCE—A REPLY

DR. W. E. CASTLE has kindly submitted to me the manuscript of his alternative explanation of my observations (1931a) on size inheritance in a mouse species cross. For the association in heredity between factors productive of a large size in several quantitative characters and a qualitative character, brown coat color, which I interpreted as genetic linkage, he offers a quite different explanation. His interpretation seems to be partly dependent on some misconceptions of my data, points which I evidently failed to make clear.

Castle states: "Green's observations show unmistakable association of larger body size and brown coat color in the back cross generation. This is shown in the significantly greater adult body weight of both sexes and in the several bone measurements and body length." The situation, as I tried to make clear, was that brown coat color was associated with larger size in several size characters,—not in *the* several. For example, skull length or width gave no clear evidence of being so associated while femur and tibia length did. I considered these differences in behavior to further indicate that not all size factors are general.

There was no intention of claiming in an earlier paper that

"larger size is a characteristic of the groups of individuals which have the third recessive character introduced from the larger race, namely non-agouti (a)." I believe I stated merely that there were indications of linkage of larger size in some of the quantitative characters and the three recessive qualitative characters studied. It was realized that linkage was by no means demonstrated, hence the repetition of the experiment under more uniform conditions.

In the Ann Arbor work (the work reported on in previous papers) the mice were born over a considerable length of time and the back cross was made in two ways, thus reducing the number in each type. In the Bar Harbor work (reported on in "Linkage in Size Inheritance") all the animals were born within a period of less than six months and were all from one type of mating. For these reasons among others it is probable that the Bar Harbor material provided the more uniform data and should be considered separately. It is from these data that I considered linkage to have been demonstrated.

It is true that I found a fairly high correlation between certain of the bone measurements but significantly higher correlations between some pairs (*e.g.*, femur—tibia) than others (*e.g.*, skull—femur). In another paper (1931) I reported on correlations of body weight with bone measurements in the pure *musculus* race. Although I believe adult weight, body length and bone lengths all to be manifestations of general size, yet they are not identical manifestations since each is influenced by local as well as general factors. The paper just cited gives reasons for this view-point.

Whether the superiority in weight at early ages of blacks over browns can best be attributed to "the initial effects of the dominant gene" as I did or to the heterozygous condition as Castle did seems to me to be largely dependent on our respective views as to the nature of heterosis. I have been inclined to adopt the purely Mendelian interpretation of inbreeding as developed largely by Shull, East and Jones. Following this, heterosis is not attributed to the heterozygous condition *per se* but to the breaking up of some of the recessive factor pairs by the introduction of dominant allelomorphs. So whether we attribute the early larger size of the heterozygous blacks to heterozygosity or to their possession of the dominant gene seems to be chiefly a matter of terminology.

The explanation which Castle substitutes for my interpretation of linkage is summarized in his statement:

> In harmony with the observations of Gates, we should expect that in the cross studied by Green the recessive genes, a, b and d, would be found together in excess of their chance frequencies. It seems clear from Green's Table I that a slightly larger body weight is also associated with this same combination of recessive genes. This does not necessitate the conclusion that larger body size is determined by genes located in the same chromosomes as a, b, and d, but only that *whatever* causes a, b, and d to go together, causes larger body size to go with them. That something is not ordinary genetic linkage (location in the same chromosome) as proved by the observations of Gates. It must be something connected with all three chromosomes and also a factor in size determination. What known structure has all these relations? Only the egg and sperm plasma which are extra-chromosomal constituents of the germ cells.

This non-chromosomal interpretation seems to rest heavily on two assumptions: (1) That the hybrids in my experiment exhibit "association systems" of chromosomes such as Gates found, and (2) that since Castle could detect no evidence of genetic linkage in his rabbit cross (1929) which involved two of my three color genes some other explanation may be in order for the mice.

As I pointed out in a preliminary communication (1930) I was unable to detect any such "association system" of chromosomes as Gates found. A complete tabulation of the total back cross and F_2 generations (the great majority of the latter from a tumor inheritance study) of the *musculus* (dba) and *bactrianus* (DBA^w) hybrids has just been made. The figures given on p. 90 show clearly that the parental genes exhibited no tendency to stay together but showed free assortment.

These figures include depleted as well as undepleted litters but separate tabulations have shown that no selective post-natal mortality exists. In the back cross the triple recessives show only an insignificant excess over the expected while on the other hand the triple dominants (*bactrianus* association) show even a slight decrease.

Were Castle's explanation correct in that whatever causes the three recessive color characters to go together (assuming that something does) also causes larger size to go with them, we should expect to find triple recessive mice larger than mice with the gene for brown in other combinations. Were my contention correct that the larger size of brown mice is due to size factors

	Total back cross		Total F_2	
	Observed	Expected	Observed	Expected
Blk. Ag. (DBA^w)	127	136.2 ± 7.4	203	204.7
dil. Blk. Ag. (dBA^w)	146	136.2 ± 7.4	70	68.2
Br. Ag. (DbA^w)	141	136.2 ± 7.4	67	68.2
Black (DBa)	136	136.2 ± 7.4	74	68.2
dil. Blk. (dBa)	110	136.2 ± 7.4	20	22.7
Brown (Dba)	132	136.2 ± 7.4	20	22.7
dil. Br. Ag. (dbA^w)	141	136.2 ± 7.4	24	22.7
dilute brown (dba)	156	136.2 ± 7.4	7	7.6
Total	1,089	1,089.6	485	485.0

linked with the gene for brown no marked differences should be encountered. If anything, the triple recessives should tend to be slightly smaller because of the piling up of recessive genes which usually are more deleterious than dominants. The following figures show that in the four quantitative characters in which significant differences were found between browns and blacks only, the triple recessives exceeded all browns in but two out of the eight contrasted pairs.

COMPARISON OF TRIPLE RECESSIVES (*Musculus* ASSOCIATION) WITH ALL BROWNS (1931a DATA)

Character		Dilute brown non-agoutis		All brown mice	
		No.	Mean	No.	Mean
Humerus length	♂♂	15	11.02 mm	72	11.10 mm
	♀♀	23	10.67 mm	77	10.74 mm
Femur length	♂♂	14	14.36 mm	71	14.39 mm
	♀♀	23	14.23 mm	76	14.24 mm
Tibia length	♂♂	15	16.25 mm	72	16.30 mm
	♀♀	23	16.03 mm	77	16.02 mm
Weight (181st day)	♂♂	15	22.2 grams	72	22.8 grams
	♀♀	23	20.8 grams	78	20.4 grams

Although Castle's rabbit cross involved two of the three genes in my mouse cross, nevertheless it was the third gene, b, which gave clearest evidence for linkage with size factors. It is quite probable, moreover, that a chromosome with a certain gene for

color might bear demonstrable size genes in one form but not in another. The chromosome with the gene for brown, it will be recalled, bears the gene for albinism in rabbits but not in mice.

A reconsideration of the data leads me still to feel that the explanation making use of genetic linkage is better borne out by the facts and involves less speculation than Castle's alternative explanation.

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TO DETERMINE GENETICAL RATIOS WHEN SELFING ORGANISMS HETEROZYGOUS FOR TWO OR MORE FACTORS

IN working out the number and constitution of all possible phenotypes and genotypes, when mating individuals heterozygous for the same factors, I have found the application of the principle of the expanded binomial, as given below, more readily understood and applied by the undergraduate student than the $3+1$ or the $3/4 \times 3/4 \times 1/4$, etc., methods as usually given in text-books on genetics. It also greatly reduces the number of calculations necessary for determining ratios, phenotypes and genotypes by means of the checkerboard method. Furthermore, the necessity of determining the exact constitution of all possible gametes, always a stumbling block for the beginning student, is eliminated.

I am venturing to outline here the method, as we have used it for some years in undergraduate classes, with the thought that it may prove helpful to other teachers of genetics.

A. In general.

1. An allelomorphic pair is represented by the binomial $a+b$.

2. In the bionomial "a" = 3 and is dominant, "b" = 1 and is recessive. a : b as 3 : 1.

B. Procedure.

1. To determine the phenotypes.

a. The number of allelomorphic pairs involved gives the power to which the binomial is to be raised. For example, AaBbCc × AaBbCc = $(a+b)^3 = a^3 + 3a^2b + 3ab^2 + b^3$.

b. The sum of the coefficients of the expanded binomial gives the number of phenotypes – 1 + 3 + 3 + 1 = 8 phenotypes.

c. For any term in the expanded binomial.

(1) The coefficient indicates the number of phenotypes,

(2) 3 raised to the power of the "a" exponent gives the number of individuals in each phenotype,

(3) An exponent gives the number of dominants or recessives in each phenotype.

For examples $3a^2b$ = (1) 3 phenotypes (2) 9 individuals in each (3) 2 dominants and 1 recessive.

d. Application, assuming 4 allelomorphic pairs.

$(a+b)^4 = a^4 + 4a^3b + 6a^2b^2 + 4ab^3 + b^4$.

1 + 4 + 6 + 4 + 1 = 16 phenotypes.

a^4 represents 1 phenotype of 81 (a^4) individuals each having 4 dominants, a^4 = 81 ABCD.

$4a^3b$ represents 4 phenotypes of 27(a^3) individuals each, each phenotype having 3 dominants and 1 recessive,

$4a^3b$ = 27 ABCd + 27 ABeD + 27 AbCD + 27 aBCD.

Similarly

$6a^2b^2$ = 9ABcd + 9AbCd + 9AbcD + 9abCD + 9aBcD + 9aBCd,

$4ab^3$ = 3Abcd + 3aBcd + 3abCd + 3abcD,

b^4 = 1 abcd.

2. To determine the genotypes.

a. In each phenotype there is one and only one individual that is homozygous for all the factors concerned. Always let the genotypes for each phenotype begin with this homozygote.

b. The number of individuals in any genotype not purely homozygous is equivalent to 2 raised to the power of the number of heterozygous dominants. Thus there are always 2 individuals when one factor is heterozygous, 4 individuals when two factors are heterozygous and so on.

c. To make sure of a full representation of genotypes, it should be noted that in each phenotype the numbers of the different combinations in each successive group of genotypes correspond to the coefficients of the binominal raised to the power of the number of dominants in the phenotype. For example the numbers of the different combinations in the successive groups of genotypes in the heterozygous phenotype ABC are 1, 3, 3, 1, the coefficients of $(a+b)^3$.

d. Application

a^4		$4a^3b$		$6a^2b^2$		$4ab^3$	b^4
81ABCD		*27ABCd*		*9ABcd*		*3Abcd*	*abcd*
AABBCCDD	—1	AABBCCdd	—1	AABBccdd	—1	AAbbccdd	aabbccdd
2AABBCCDd	—4	2AABBCcdd	—3	2AABbccdd	—2	2Aabbccdd	
2AABBCcDD		2AABbCCdd		2AaBBccdd			
2AABbCCDD		2AaBBCCdd		4AaBbccdd	—1	*3aBcd*	
2AaBBCCDD		4AABbCcdd	—3			aaBBccdd	
4AABBCcDd		4AaBBCcdd		*9AbcD*		2aaBbccdd	
4AABbCCDd		4AaBbCCdd		AAbbccDD	—1		
4AABbCcDD	—6	8AaBbCcdd	—1	2AAbbccDd	—2	*3abCd*	
4AaBBCCDd				2AabbccDD		aabbCCdd	
4AaBBCcDD		*27ABcD* etc.		4AabbccDd	—1	2aabbCcdd	
4AaBbCCDD		AABBccDD	—1				
8AABbCcDd	—4	2AABBccDd	—3	*9abCD* etc.		*3abcD*	
8AaBBCcDd		2AABbccDD		aabbCCDD	—1	aabbccDD	
8AaBbCCDd		2AaBBccDD		2aabbCcDD	—2	2aabbccDd	
8AaBbCcDD		4AABbccDd		2aabbCCDd			
16AaBbCcDd	—1	4AaBBccDd	—3	4aabbCcDd	—1		
		4AaBbccDD					
		8AaBbccDd	—1				

The points to bear in mind do not lend themselves to simple statements and seem rather formidable in the first reading. When once applied, however, the process becomes thereafter very simple for any number of allelomorphic pairs. The statement has been written out very fully in the interest of the undergraduate, not for the expert.

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DOMINANT EYE-COLORS IN DROSOPHILA

DOMINANT eye-color mutations that have been produced by x-ray and radium irradiation of *Drosophila* have been found to be associated with some type of chromosomal aberration (Weinstein, 1928, Muller, 1930, Oliver, 1930, and Hanson and Winkleman, 1929). The eye-color is usually non-homogeneous and eversporting. In the fall of 1930, additional dominant eye-colors, which the author obtained by x-raying, were examined genetically and cytologically for the purpose of finding the possible relationships between the chromosome abnormality and the expression of the dominant eye-color.

Normal males were given an x-ray dosage of approximately 4,000 r units (52 kilovolts, 5 milliamperes, 20 cm distance from tube, 1 mm thickness of aluminum, and exposure time of 2.5 hours). Offspring of treated males mated to untreated attached-X yellow females were examined for departures from the

normal type. Seven dominant eye-color mutations were found among 20,929 F_1 individuals.

Two of the dominant eye-color mutations are homogeneous and stable. The first, Salmon, is associated with a mutual translocation involving approximately half of the second and half of the third chromosome. Cross-over classes furnish strong evidence that the eye-color mutation is located exactly at the point of breakage of one of the involved chromosomes. Cytological examination of dividing oogonial cells shows that both arms of the composite second and third chromosomes exhibit a strong tendency to pair with their homologues in the normal set of autosomes.

The homogeneous eye-color, Henna, a brownish-red, appeared in a fly carrying a translocation from the third to the second chromosome. However, the mutation proved to be entirely independent of the translocation, and behaved as if it were a gene mutation located near hairy on the third chromosome. It is believed that Henna is the first dominant eye-color mutation in *Drosophila* reported as definitely due to a gene mutation.

With the five non-homogeneous or eversporting eye-colors, called Dilute eyes, the normal red color appears diluted in some of the ommatidia of the compound eye. Cream-eye, obtained by exposure to radium (Hanson and Winkleman, 1929), is included in the study of the Dilute eye-colors, since it shows peculiarities similar to those observed with the "Dilutes" produced with x-rays. The eye has a cream color only when the individual is homozygous or hemizygous for vermilion at the same time that it carries the dominant eye-color ($Dilute_1$).

Usually all the ommatidia are affected in $Dilute_1$ and $Dilute_2$ and the eye is of a uniformly diluted red color. (The color of $Dilute_1$ has been made the subject of a preliminary quantitative study by spectrographic methods, E. A. and L. C. Van Atta, 1931.) Both eye-colors involve the same type of inversion in the second chromosome. A comparison of the cross-over classes and cytological configurations leads to the conclusion that the left arm of the second chromosome has become transferred to the end of the right arm, making a rearranged chromosome with a terminal rather than a median spindle fiber attachment. The $Dilute_3$ eye has a characteristically mottled appearance and is associated with an inversion in the right arm of the second chromosome. In $Dilute_4$ and $Dilute_5$ only a few of the omma-

tidia of the eye are affected, and non-reciprocal translocations between the second and third chromosomes are present. There are inversions in the second chromosome of $Dilute_6$, with small translocations from the second to the third and fourth chromosomes. Frequent non-disjunction results in the presence of three fourth chromosomes in oogonial cells. Several types of hyperploid individuals survive.

Results from tests with Bridges' counterbalanced stock of the Pale-translocation show that all the non-homogeneous eye-color mutations are located in the extreme right end of the second chromosome. The expression of the dominant eye-color is suppressed in all cases by the normal allelomorphs in the homologous second chromosome and the translocated section of the Pale stock which includes the loci of plexus and speck. That the Dilute eye-colors form an allelomorphic series in this region seems still more probable from the fact that there are no viable combinations of any of the Dilute eye-colors. This was shown by matings of Dilute Curly flies using all possible combinations of Dilute eyes.

The findings for Salmon and Henna have demonstrated that the dominant mutations in eye-color are not necessarily associated with eversporting behavior or chromosomal aberration. However, if the eversporting tendency is present, it is accompanied by some sort of chromosomal rearrangement, as seen in the Dilute eyes. These have all resulted from mutation in the extreme right end of the second chromosome at a locus that is evidently especially susceptible to mutations of this type.

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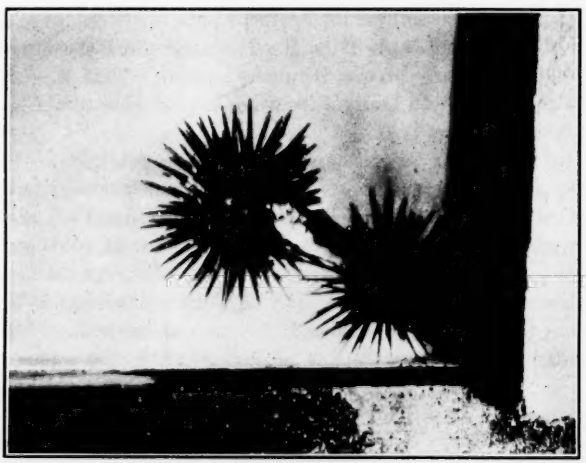
ON CERTAIN FEEDING HABITS OF THE SEA-URCHIN ARBACIA

THE sea-urchin, *Arbacia punctulata,* is generally regarded as an omnivorous scavenger, evidence of which is seen in its fecal detritus. It feeds for the most part on the remains of animals and plants that collect on the sea-bottom. But it is not always limited to so tame a diet. Occasionally, it rises in its quest for food to almost sportsmanlike activities.

If a number of sea-urchins are put in an aquarium in which there is a goodly supply of mummichugs, *Fundulus heteroclitus,*

one or more of these fishes may be caught and eaten by the sea-urchins. The capture usually takes place at night, and the prey is almost always a partly spent fish. I doubt if a fully vigorous Fundulus is ever taken by a sea-urchin. I have never witnessed the first steps in the capture. What I have seen, on first arrival, is a fish, usually a small one, whose head or tail is largely covered by the sea-urchin. The spines and ambulacral feet of the captor are used to hold the prey against either the bottom or the sides of the aquarium, while the jaws of the sea-urchin are vigorously plied on the flesh of the fish. Not infrequently, a fish once caught is beset by a second sea-urchin and the two together complete the demolition of the Fundulus, as shown in the accompanying figure. The fish, when caught, often offers some resistance, but



Glass wall of an aquarium against which a Fundulus is being held by two sea-urchins, *Arbacia punctulata*, which are devouring the fish. Photograph by Dr. E. C. Cole.

it soon succumbs to the sea-urchin as though it had been poisoned by its captor. However, I have no direct evidence that the sea-urchin can exert such influences. The main point that this note is intended to record is the capture of live fish by the sea-urchin, *Arbacia punctulata*.

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